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(54) Method for obtaining modified immunoglobulins with reduced immunogenicity of murine antibody variable domains, compositions containing them

(57) Modified chimaeric antibodies, and antibody heavy and light chains, which comprise variable domains derived from a first mammalian species, usually mouse, and constant domains from a second mammalian species, usually human. Modification concerns the variable domains, in particular the framework regions of the variable domains. The modifications are made only in T-cell antigenic structures present in framework regions, and do not cover canonical structures or Vernier zone. The modifications adapt the amino acid sequences concerned to those occurring in corresponding antibodies derived from said second mammalian species. Thus, the modified chimaeric antibodies retain the original antigen recognition and binding properties but become less immunogenic to said second mammalian species, which improves their therapeutical utility with said second mammalian species. Recombinant DNA technology may be used to construct and produce the modified chimaeric antibodies.

FIGURE 1: DEDUCED AMINO ACID SEQUENCES

A VK OF MURINE R3 ANTIBODY

D V L M T Q I P L S L P V S L G D Q A S I S C R S S Q
N I V H S N G N T Y L Q W Y L Q K P G Q S P N L L
I Y K V S N R F S G V P D R F R G S G S G T D F T L K
I S R V E A E D L G V Y Y C F Q G S H V P W T F G G G
T K L E I K R A

B VH OF MURINE R3 ANTIBODY

Q V Q L Q Q P G A E L V K P G A S V K L S C K A S G Y
T F T N Y Y I Y W V K Q R P G Q G L E W I G G I N P T
S G G S N F N E K F K T K A T L T V D E S S T T A Y M
Q L S S L T S E D S A V Y Y C T R Q G L W F D S D G R
G F D Y W G Q G T T L T V S S

EP 0 699 755 A2

Description

FIELD OF THE INVENTION

The present invention is related to the field of immunology, in particular to a method for obtaining modified immunoglobulins with reduced immunogenicity of murine antibody variable domains and compositions containing them.

BACKGROUND OF THE INVENTION

The immune system builds antibodies that bind to a vast range of antigens with high avidity and specificity, and trigger effector mechanisms. Antibodies have been used in medicine as diagnostic and therapeutic agents, and their potential has been successively enhanced with the advent of new technologies.

Hybridoma technology allowed isolation of cell lines secreting antibodies of a single specificity (Köhler G., Milstein C. (1975) Nature (London) 256, 495-497), and gene technology has allowed the construction of a range of engineered antibodies from hybridomas.

Engineering of antibodies is facilitated by their domain structure and may further improve the utility of many antibodies by the acquisition or loss of some of their properties. The antigen-binding properties of the antibody provide the recognition function and this can be attached to one or more of a number of effector agents. The combination of these two features must then be tested against the criteria of efficacy, specificity and immunogenicity.

Monoclonal antibody producing hybridomas have been most readily obtained from immunized rodents. At present the use of several murine monoclonal antibodies has been widespreaded for the imaging and treatment of malignancy, prophylactic administration to guard against toxic shock, modification of graft rejection episodes, and to temper acute inflammatory reactions.

In most of the cases where rodent antibodies have been used for therapy, the recipients have elicited an immune response directed towards the antibody. These reactions have limited the duration and effectiveness of the therapy.

Development of similar reagents from human sources has been frustated, although several options exist, using for example SCID-hu mice, in vitro immunization, recombinatorial libraries, or some useful combination of these. Because there are many well-characterized rodent monoclonal antibodies already available which might be used in the clinic if the immune response could be abolished, the production of engineered antibodies has received much attention.

Engineered antibodies have been designed to replace as much as possible of the xenogeneic sequences with the equivalent human sequence. Among the genetically engineered antibodies are chimaeric antibodies in which segments from immunoglobulins from diverse species are joined together.

Initially, chimaeric antibodies were constructed containing the rodent variable regions fused to human constant domains. Particularly mouse/human chimaeric antibodies are potentially useful for immunotherapy for they should exhibit the same specificity but reduced immunogenicity compared to their murine counterparts. The following references describe chimaeric antibody technology: Lobuglio et al, Proc. Natl. Acad. Sci. USA 86: 4220-4224 (1989); United States Patent 4,816,567; PCT International Publication No. WO 87/02671 published May 7, 1987; European Patent Publication No. 255,694 published February 10, 1988; European Patent Publication No.274,394 published July 13, 1988; European Patent Publication No. 323,806 published July 12, 1989; PCT International Publication No. WO 89/00999 published February 9, 1989; European Patent Publication No. 327,000 published August 9, 1989; European Patent Publication No. 328,404 published August 16, 1989; and European patent Publication No. 332,424 published September 12, 1989.

It is worth noting that even the replacement of the constant regions with human equivalents may not effectively reduce their immunogenicity. Still approximately half of the recipients mounted an immune response to the rodent variable regions. Subsequently, rodent antibodies have been extensively manipulated to resemble more fully human antibodies.

Further reduction in the immunogenicity of chimaeric antibodies has been achieved by grafting only the complementarity determining regions (CDRs) from the rodent monoclonal antibody onto human framework regions (FRs) prior to its subsequent fusion with an appropriate constant domain (Jones et al, Nature 321: 522-525 (1986)). This procedure to accomplish CDR-grafting often results in imperfectly humanized antibodies, it means, the resultant antibody has either lost affinity or in an attempt to retain its original affinity a number of the murine framework residues have replaced the corresponding ones of the chosen human framework (Winter, European Patent Application, Publication No. 239,400; Riechmann et al, Nature 332: 323-327 (1988)).

A number of strategies has been developed with the objective of identifying the minimum number of residues for transfer to achieve a useful binding affinity with the least potential consequences on immunogenicity. However, it has emerged that each of these strategies has only been successful to some degree in the reconstitution of parental affinity.

The ligand binding characteristics of an antibody combining site are determined primarily by the structure and relative disposition of the CDRs, although some neighbouring framework residues also have been found to be involved in antigen binding (Davies et al, Ann. Rev. Biochem. 59: 439-473 (1990)). Thus, the fine specificity of an antibody can be preserved if its CDR structures and some of the neighbouring residues, their interaction with each other, and their interaction with the rest of the variable domains can be strictly maintained.

A further procedure for the humanization of an antibody has been suggested by Padlan (Padlan, European Patent Application, Publication No. 0 519 596 A1; Padlan, Molecular Immunology 28: 489-498 (1991)). It is based on the fact that the antigenicity of a protein is dependent on the nature of its surface, and a number of the solvent-accessible residues in the rodent variable region are substituted by residues from a human antibody. The locations of these residues are identified from an inspection of the high resolution X-ray structures of the human antibody KOL and the murine antibody J539. The choice of the human surface residues is arrived at by identifying the most homologous antibody sub-group.

The nature of the protein surface is important for its recognition and internalization by antigen-processing cells, specifically by antigen-specific B-cells. In addition, the recognition of specific linear sequences by T-cells is also an important element in the immunogenicity of proteins.

Several groups have developed automated-computerized methods for the identification of sequence features and structural determinants that play a role in the MHC restriction of helper T-cell antigenic peptides (Bersofsky et al, J. Immunol. 138: 2213-2229 (1987), Elliott et al, J. Immunol. 138: 2949-2952 (1987), Reyes et al, J. Biol. Chem. 264: 12854-12858 (1989)). Using these algorithms, it has been possible to identify predicted T cell-presented peptides.

Analysis of antibodies of known atomic structure has elucidated relationships between the sequence and three-dimensional structure of antibody combining sites (Chothia et al, J. Biol. Chem. 196: 901-917 (1987)). These relationships imply that, except for the third region in the VH domains, binding site loops have one of a small number of main-chain conformations: "Canonical structures". The canonical structure formed in a particular loop is determined by its size and the presence of certain residues at key sites in both the loop and in framework regions.

An additional subset of framework residues has been defined as "Vernier" zone, which may adjust CDR structure and fine-tune the fit to antigen (Foot et al, J. Mol. Biol. 224: 487-499 (1992)). Substitutions of these residues have been shown to be important to restoring the affinity in CDR grafted antibodies, so the Vernier zone has an obvious consequence for the design of humanized antibodies.

SUMMARY OF THE INVENTION

It is, accordingly, an objective of the present invention to provide a means of converting a monoclonal antibody of one mammalian species to a monoclonal antibody of another species. Another object is to predict potential T-epitopes within the sequence of variable regions. Another object is to identify the amino acid residues responsible for species specificity or immunogenicity within the sequence of the monoclonal antibody responsible of the T-immunogenicity. Another object is to judiciously replace the amino acid residues within the T-epitope sequences of one species with those of a second species so that the antibodies of the first species will not be immunogenic in the second species. A further object is to make replacements only in the framework regions of the heavy and light chains and not in the complementarity determining regions; also the amino acids belonging to the Vernier zone and those involved in the canonical structures cannot be replaced. Another object of the invention is to provide novel DNA sequences incorporating the replacement amino acid residues. Another object is to provide a vector containing the DNA sequences for the altered antibody. Another object is to provide a eukaryotic or procaryotic host transformed with a vector containing the DNA sequence for the modified antibody.

A unique method is disclosed for identifying and replacing amino acid residues within T-cell antigenic sequences which converts immunoglobulin antigenicity of a first mammalian species to that of a second mammalian species. The method will simultaneously change immunogenicity and strictly preserve ligand binding properties. A judicious replacement of those amino acid residues within T-cell antigenic sequences of the variable regions, which are not involved in the three-dimensional structure, has no effect on the ligand binding properties but greatly alters immunogenicity.

BRIEF DESCRIPTION OF THE DRAWINGS.

FIGURE 1: Deduced amino acid sequence of (a) VK and (b) VH of murine R3 antibody. CDRs are underlined.
FIGURES 2 and 3: Analysis for the modification of the variable regions of heavy and light chains of antibody IOR-R3.

A: sequence of the variable region of the murine IOR-R3 monoclonal antibody.
B: sequence of the variable region of the most homologous human immunoglobulin.
C: sequence of the modified variable region of IOR-R3.

shading: predicted T-cell antigenic sequences.
underlined amino acid residues: amino acids involved in tertiary structure.
bold font: complementarity determining regions.
amino acid residues in boxes: proposed replacements.
The description is the same for both, heavy and light chains.

FIGURE 4: Molecular model of the variable region of mAb R3 displayed as a ribbon. VH is on the right and is darker than VL. The model shows the side chain of murine residues that were mutated in order to humanize the predicted amphipatic segments.

FIGURE 5: Detection of binding of the chimaeric and mutant R3 to EGF-R by RRA.
Antigen binding activity was assayed in different concentrations of purified murine R3 (-.-), chimaeric R3 (+) and mutant VHR3/muR3VK (*) and plotted as CPM of bound ^{125}I-EGF against log of the concentration of each antibody. (concentration of IgG was quantitated by ELISA.)

FIGURE 6: Immunization of monkeys with murine R3, chimaeric R3 and mutant R3.
ordinates: Absorbance at 405 nm.
abscises: number of days of blood collected.
The ELISA was performed as described in example 9. The arrows indicate the time of intravenous injection of 2 mg of each mAb. The serum dilution used was 1 / 10 000.

FIGURES 7 and 8: Analysis for the modification of the variable regions of heavy and light chains of antibody IOR-T1.

A: sequence of the variable region of the murine IOR-T1 monoclonal antibody.
B: sequence of the variable region of the most homologous human immunoglobulin.
C: sequence of the modified variable region of IOR-T1 antibody.

The symbols are the same as in FIGURE 2. The description is the same for both, heavy and light chains.

FIGURES 9 and 10: Analysis for the modification of the variable regions of heavy and light chains of antibody IOR-CEA1.

A: sequence of the variable region of the murine IOR-CEA1 monoclonal antibody.
B: sequence of the variable region of the most homologous human immunoglobulin.
C: sequence of the modified variable region of IOR-CEA1 antibody.

The symbols are the same as in FIGURE 2. The description is the same for both, heavy and light chains.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a procedure which simultaneously reduces immunogenicity of the rodent monoclonal antibody while preserving its ligand binding properties in its entirety. Since the antigenicity of an immunoglobulin is dependent on the presence of T-cell antigenic peptides within its sequence, the immunogenicity of a xenogenic or allogenic antibody could be reduced by replacing the residues included in the T-cell antigenic sequences which differ from those usually found in antibodies of another mammalian species.

The replacement of residues does not include those involved in the canonical structures or in the Vernier zone. This judicious replacement of residues has no effect on the structural determinants or on the interdomain contacts, thus, ligand binding properties should be unaffected as a consequence of alterations which are limited to the variable region framework residues.

(1) Analysis of homology of variable regions

The present procedure makes use of the available sequence data for human antibody variable domains compiled by Kabat et al, "Sequences of proteins of Immunological Interest", Fifth edition, Bethesda, Maryland; National Inst. of Health, 1994.

In the first step the variable domains of any heavy or light chain of a first animal species, e.g. the mouse, are compared with the corresponding variable domains of a second animal species, e.g. human. It is intended that this invention will allow the antigenic alteration of any animal species antibody.

The comparison is made by an automated-computerized method (PC-DOS HIBIO PROSIS 06-00, Hitachi). The most homologous human variable regions are then compared, residue for residue, to the corresponding murine regions. This will also define the human subgroup to which each mouse sequence most closely resembles.

(2) Prediction of T-epitopes

In the second step, the two homologous variable region sequences, mouse and human, are analysed for the prediction of T-antigenic sequences.

The algorithm AMPHI (Bersofsky et al, The Journal of Immunology 138: 2213-2229 (1987)) predicts a Helical sequences. The algorithm SOHHA predicts the strip of helix hydrophobicity (Elliott et al, J. Immunol. 138: 2949-2952 (1987)). These algorithms predict T-cell presented fragments of antigenic proteins.

(3) Analysis for immunogenicity reduction

Those residues in the mouse framework which differ from its human counterpart are replaced by the residues present in the human counterpart. This switching (replacement) occurs only with those residues which are in the T-antigenic sequences.

Finally, replacement of those residues responsible for the canonical structures or those involved in the Vernier zone could have a significant effect on the tertiary structure. Hence, they cannot be included in the replacement. Additional information about the influence of the proposed replacements on tertiary structure or the binding site could be obtained from a molecular model of the variable regions.

The molecular model can be built on a Silicon Graphics Iris 4D workstation running UNIX and using the molecular modeling package "QUANTA" (Polygen Corp.).

(4) Method for constructing and expressing the altered antibody

The following procedures are used to prepare recombinant DNA sequences which incorporate the CDRs of a first mammalian species, usually animal, e.g. murine mAb, both light and heavy chains, into a second mammalian species, preferably human, appearing frameworks that can be used to transfect mammalian cells for the expression of recombinant antibody less immunogenic and with the antigen specificity of the animal monoclonal antibody.

The present invention further comprises a method for constructing and expressing the modified antibody comprising:

a.-) mutagenesis and assembly of variable region domains including CDRs and FRs regions. The PCR-mutagenesis method (Kamman et al, Nucleic Acids Res. 17: 5404-5409 (1989)) is preferably used to introduce the changes at different positions.

b.-) preparation of an expression vector including one variable region and the corresponding human constant region which upon transfection into cells results in the secretion of protein sufficient for affinity and specificity determinations.

c.-) co-transfection of heavy and light chain expression vectors in appropriate cell lines.

After about 2 weeks, the cell supernatants are analyzed by ELISA for human IgG production. The samples are then analysed by any method for human IgG capable of binding to specific antigens.

The present invention provides a method for incorporating CDRs from animal monoclonal antibodies into frameworks which appear to be human immunoglobulin in nature so that the resulting recombinant antibody will be either weakly immunogenic or non-immunogenic when administered to humans. Preferably the recombinant immunoglobulins will be recognized as self proteins when administered for therapeutic purpose. This method will render the recombinant antibodies useful as therapeutic agents because they will be either weakly immunogenic or non-immunogenic when administered to humans.

The invention is further contemplated to include the recombinant conversion of any animal monoclonal antibody into a recombinant human-appearing monoclonal antibody by providing that with a suitable framework region.

The invention is intended to include the conversion of any animal immunoglobulin to a human-appearing immunoglobulin. It is further intended that human-appearing immunoglobulin can contain either Kappa or Lambda light chains or be one of any of the following heavy chain isotypes (alpha, delta, epsilon, gamma and mu).

The following examples intend to ilustrate the invention but not to limit the scope of the invention.

EXAMPLE 1: Murine Variable region of R3 monoclonal antibody DNA sequencing

Cytoplasmic RNA was extracted from about 10^6 R3 (anti Epidermal growth Factor receptor) hybridoma cells as described by Faloro et al (Faloro, J. et al, Methods in Enzymology 65: 718-749, 1989).

The cDNA synthesis reaction consisted of 5 ug RNA, 50 mM Tris-HCl, pH 7.5, 75 mM KCl, 10 mM DTT, 3 mM $MgCl_2$, 25 pmol of CG2AFOR primer (5' GGAAGCTTAGACCGATGGGGCCTGTTGTTTTG 3') for the heavy chain variable region or CK2FOR primer (5' GGAAGCTTGAAGATGGATACAGTTGGTGCAGC 3') for the light chain variable region, 250 uM each of dATP, dTTP, dCTP, dGTP, 15 U ribonuclease inhibitor (RNA guard, Pharmacia) in a total volume of 50 ul. Samples were heated at 70°C for 10 min and slowly cooled to 37°C over a period of 30 min. Then, 100 units MMLV reverse transcriptase (BRL) were added and the incubation at 37°C continued for 1 hour.

The VH and VK cDNAs were amplified using the PCR as described by Orlandi et al (Orlandi, R. et al, Proc. Natl. Acad. Sci. USA 86: 3833-3837, 1989). For PCR amplification of VH, DNA/primer mixtures consisted of 5 ul cDNA, 25 pmoles of CG2AFOR primer (5' GGAAGCTTAGACCGATGGGGCCTGTTGTTTTG 3') and VH1BACK primer (5' AGGT(G/C)(A/C)A(A/G)CTGCAG(G/C)AGTC(A/T)GG 3').

For PCR amplification of VK, DNA/primers mixtures consisted of 5 ul cDNA and 25 pmoles of CK2FOR primer (5' GGAAGCTTGAAGATGGATACAGTTGGTGCAGC 3') and VK10BACK primer (5' TTGAATTCCAGTGATGTTTTGATGACCCA 3'). To these mixtures were added 2.5 mM each of dATP, dCTP, dTTP, and dGTP. 5 ul constituents of 10X

buffer thermolase and 1 unit of Thermolase (IBI) in a final volume of 50 ul. Samples were subjected to 25 thermal cycles at 94°C, 30 sec; 50°C, 30 sec; 72°C, 1 min; and a last incubation for 5 min at 72°C. Amplified VH and VK DNA were purified on Prep. A Gene purification kit (BioRad).

The purified VH and VK cDNA were cloned into M13 vector. Clones were sequenced by the dideoxy method using T7 DNA Pol (Pharmacia). See figure 1.

EXAMPLE 2: Construction of chimaeric genes

We reamplified the cDNA by PCR using VH1BACK primer (5' AGGT(G/C)(A/C)A(A/G)CTGCAG(G/C)AGTC(A/T)GG 3') and VH1FOR primer (5' TGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAG 3') for VH and VK3BACK primer (5' GACATTCAGCTGACCCA 3') and VK3FOR primer (5' GTTAGATCTCCAGTTTGGTGCT 3') for VK. The amplified cDNAs were digested with PstI and BstEII for the VH gene or PvuII and BglII for the VK gene. The fragments were cloned into M13-VHPCR1 (digested with PstI and BstEII) or into M13-VKPCR1 (digested with PvuII and BclI). Details of vectors are given by Orlandi, R. et al, Proc. Natl. Acad. Sci. USA 86: 3833-3837, 1989. The M13VHPCR-R3 and M13VKPCR-R3 containing V gene inserts were identified directly by sequencing.

The VH gene together with the Ig heavy chain promoter, appropriate splicing sites and signal peptide sequences were excised from M13 vectors by digestion with HindIII and BamHI and cloned into an expression vector (pSVgpt). A human IgG1 constant region (Takahashi, N. et al, Cell 29: 718-749, 1982) was then added as a BamHI fragment. The resultant construction was R3VH-pSVgpt. The construction of the R3VK-pSVhyg was essentially the same except that the gpt gene was replaced by the hygromicin resistance gene and a human Kappa chain constant region was added (Hieter, P.A. et al, Cell 22: 197-207, 1980).

EXAMPLE 3: Modification of the variable domain sequences of IOR-R3 murine monoclonal antibody to humanize the predicted T-cell antigenic sequences

The variable region sequences of heavy and light chains of R3 were analyzed for T-cell antigenic sequences. It was made by using the computer algorithm AMPHI, which predicts segments of the sequences 11 amino acids in length with an amphipatic helix structure, that is have one side hydrophobic and one side hydrophilic which bind to MHC II molecules.

Within the variable domain sequence of the heavy chain were predicted 5 segments which are (using Kabat's numbering):

1. FR1 between amino acids 3-13.
2. FR1 between amino acids 8-20.
3. FR2 and CDR2 between amino acids 39-55.
4. FR3 between amino acids 74-84.
5. FR4 and CDR3 between amino acids 100c-110.

Figure 2 shows the sequences corresponding to the heavy chain.

This murine sequence is compared with the immunoglobulin sequences included in the GeneBank and EMBL database. The most homologous human variable region sequence is determined and also the human subgroup to which the murine sequence most closely resembles is defined. In this case the human sequence found was a fetal immunoglobulin called HUMIGHVA, which variable region has 75% of homology with the FR regions of the murine immunoglobulin R3.

Both variable region sequences, human and murine are then compared, residue for residue, and those residues in FR regions which are not involved in the vernier zone or with the canonical structures are selected. Therefore they could be changed by those residues at the same position within the human sequence.

Finally, this analysis is enriched with computer modeling of the binding site. On the molecular model it is possible to define those replacements which will perturb the tertiary structure of the binding site.

For the heavy chain of murine R3 we propose 6 replacements:

1. LEU at position 11 by VAL
2. VAL at position 12 by LYS

With only these two replacements it is possible to disrupt the amphipatic helix and therefore the predicted T-epitope in the FR1.

3. SER at position 75 by THR
4. THR at position 76 by SER
5. ALA at position 78 by VAL
6. THR at position 83 by ARG

In this case, with the replacements proposed in the FR3, it is humanized.

The T-cell antigenic sequence in the FR2 contains two PRO which is a very rare amino acid residue in most of the helical antigenic sites, so we propose that it is not a real T-cell epitope.

In the position 108 at the FR4 appears THR which is present in the same position in some human immunoglobulins, only residue 109 (LEU) is very rare in human, except for this point difference most of the predicted T-cell epitope is human, on this basis it does not need to be modified.

In Figure 3 the analysis for the light chain of murine R3 is shown.

In the sequence only one amphipatic helix was predicted, between residue 52-63 corresponding to CDR2 and FR3, and in this region only one point difference exists between murine and human sequences, at position 63. No replacement is proposed, because this murine light chain should be non-immunogenic in human (see molecular modelling).

EXAMPLE 4: Molecular Modelling of mAb R3 VK and VH

A model of the variable regions of mouse mAb R3 was built using the molecular modeling program QUANTA/CHARm 4.0 (Molecular Simulations Inc., 1994), running on a 150 MHz Silicon Graphics Indigo Extreme workstation. The VK and VH frameworks were built separately from Fab 26-10 (Jeffrey, P.D et al, Proc. Natl. Acad. Sci. USA 90, 10310, 1993) and Fab 36-71 (Strong, R.K. et al, Biochemistry 30, 3739, 1993), respectively. Fab 26-10 and mAb R3 have 92% homology in the VK frameworks and 88% homology in the whole VK region. The VH frameworks of Fab 36-71 and mAb R3 have 85% homology.

Coordinates were taken from the Brookhaven Protein Data Bank (entries 1IGI and 6FAB). The frameworks of Fab 36-71 were fitted to the frameworks of Fab 26-10, matching only those residues that have been found to be often involved in the interface between the light and heavy variable regions (Chotia, C. et al, J. Mol. Biol. 186, 651, 1985). The VH domain of Fab 26-10 and the VK domain of Fab 36-71 were then deleted leaving the needed hybrid. Side-chain replacements were performed following the maximum overlap procedure (Snow, M.E. et al, Proteins 1, 267, 1986) and comparing, where possible, with other crystal structures.

The hypervariable regions of the R3-Variable Light (VL) domain (L1, L2 and L3) were built retaining the same main-chain conformations as in Fab 26-10, since the corresponding CDRs in both antibodies are highly homologous and belong to the same canonical structural groups (Chotia, C. et al, Nature 342, 877, 1989). In the VH domain of mAb R3, CDR H1 belongs to canonical structural group 1, as in Fab 36-71, so the main-chain torsion angles of the parent molecule were kept. CDR H2 corresponds to canonical structural group 2 and the main-chain conformation for this loop was taken from the Fv fragment 4D5 (entry 1FVC), which was selected among other highly resolved structures because of the good matching of its H2 loop base with the framework of Fab 36-71. For all the above mentioned loops comparisons with other CDRs from the Data Bank were made to orient the side chains.

To model CDR H3, which in mAb R3 was 14 amino acids long, a high temperature molecular dynamics was used for conformational sampling (Bruccoleri, R.E. et al, Biopolymers 29, 1847, 1990). First, the whole structure without CDR H3 was subjected to an energy minimization keeping residues H-94 and H-103 fixed and using harmonic constraints of 10 Kcal/(mole atom A^2) for main chain atoms. Then a loop was constructed with an arbitrary conformation starting from the two previously fixed amino acids. Those residues close to the framework were placed taking into consideration other crystal structures and the top part of the loop was built with an extended conformation avoiding strong steric interactions with the rest of the molecule. For the next modeling steps only CDR H3 and the neighbouring side chains within a distance of $5A^0$ were permitted to move. An energy minimization was first carried out and then a molecular dynamics at 800 K was run for 150 picoseconds. The time step for the run was set to 0.001 picosecond and coordinates were saved every 100 steps. The 120 lowest energy conformations from the dynamics run were extracted and subjected to an energy minimization in which all atoms in the structure were allowed to move. Several low-energy conformations were obtained and the one with the lowest energy was used in the subsequent analyses. Differences between murine and humanized variants of R3 antibody were individually modeled to investigate their possible influence on CDR conformation.

Amino acid replacements in positions 11, 12 (FR1) and 83 (FR3) in the heavy chain variable region are quite enough distant from the CDRs-FRs boundaries and should not have any influence on binding affinity. SER 75 residue is pointing to outside, thus the replacement by THR seems not to be important for binding capacity. By contrary THR 76 is accessible from the top of the molecule and could be involved in the interaction with the antigen. But the substitution of THR 76 by SER is a conservative change, leading to no major variations in binding affinity probably.

The replacement of ALA 78 by VAL should not require steric rearrangements. However VAL 78 could "push" forward ILE 34 (HI). In general, the proposed point mutations should not affect binding affinity according to the computer-aided molecular modelling study (Figure 4).

The same analysis was done in the light chain variable region of IOR-R3, molecular modelling indicates it is not necessary to make any changes in this region.

EXAMPLE 5: Construction of mutant heavy chain variable region of R3 by PCR mutagenesis

The changes in the amino acids of mutant heavy chain variable region were constructed using PCR mutagenesis (Kammann, M. et al, Proc. Natl. Acad. Sci. USA 86, 4220-4224, 1989).

Briefly: Two amplification by PCR: the reaction mixture was: 0.5 ul the VH supernatant of single strand DNA cloned in M13, 25 pmoles mutagenic oligo 1 or 2, 25 pmoles mutagenic oligo 3 or 4 primers (See below the primers sequences). To these mixtures were added 2.5 mM each of dATP, dCTP, dTTP, and dGTP, 5 ul constituents of 10X Vent Polymerase buffer (NEB) and 1 unit of Vent DNA Polymerase (NEB) in a final volume of 50 ul. Samples were subjected to 12-15 thermal cycles at 94°C, 30 sec; 50°C, 30 sec; 75°C, 1 min; and a last incubation for 5 min at 75°C. The products of both PCRs are joined in a second PCR using the outside primers only (3 and 4). Amplified VH DNA was purified on Prep. A Gene purification kit (BioRad).

For the changes in the FR1 of LEU 11 and VAL 12 by VAL and LYS, respectively, the following primers were used:
Primer 1: 5' GAAGCCCCAGGCTTCTTCACTTCAGCCCCAGGCTG 3'.
Primer 3: 5' GTAAAACGACGGCCAGT 3'.

These primers are combined in one PCR.
Primer 2: 5' CAGCCTGGGGCTGAAGTGAAGAAGCCTGGGGCTTCA 3'.
Primer 4: 5' ACTGGCCGTCGTTTTAC 3'.

These primers are combined in one PCR.

Then, the products of both PCRs are combined in one PCR using primers 3 and 4.

For the changes in the FR3, SER 75, THR 76, VAL 78 and THR 86 by THR, SER, VAL and ARG, respectively, the following primers were designed:
Primer 1: 5' GCAGAGTCCTCAGATCTCAGGCTGCTGAGTTGCATGTAGACTGTGCTGGTGGATTCGTCTACCGT 3'.
Primer 3: 5' GTAAAACGACGGCCAGT 3'.

These primers are combined in one PCR.
Primer 2: 5' ACGGTAGACGAATCCACCAGCACAGTCTACATGCAACTCAGCAGCCTGAGATCTGAGGACTCTGC 3'.
Primer 4: 5' ACTGGCCGTCGTTTTAC 3'.

These primers are combined in one PCR.

Then, the products of both PCRs are combined in one PCR using primers 3 and 4.

After mutagenesis VH genes were cloned in expression vectors (pSVgpt) yielding the plasmids R3 mut VH-pSVgpt.

EXAMPLE 6: Transfection of DNA into NSO cells

Four ug of R3VH-pSVgpt and 8 ug R3VK-pSVhyg (chimaeric) or R3 mutant VH-pSVgpt and murine R3VK-pSVhyg were linearized by digestion with PvuI. The DNAs were mixed together, ethanol precipitated and dissolved in 25 ul water. Approximately 10^7 NSO cells (Rat myeloma NSO is a non-Ig secreting cell line) were grown to semiconfluency, harvested by centrifugation and resuspended in 0.5 ml DMEN together with the digested DNA in an electroporation cuvette. After 5 min on ice, the cells were given a single pulse of 170V at 960 uF (Gene-Pulser, Bio-Rad) and left in ice for a further 30 min. The cells were then put into 20 ml DMEN plus 10% fetal calf serum and allowed to recover for 24 hours. At this time the cells were distributed into a 96-well plate and selective medium applied, transfected clones were visible with the naked eyes 14 days later.

EXAMPLE 7: Quantification of IgG production

The presence of human antibody in the medium of wells containing transfected clones was measured by ELISA. Microtiter plate wells were coated with goat anti-human IgG (heavy chain specific) antibodies (Sera-Lab). After washing with PBST (phosphate buffered saline containing 0.02% Tween 20, pH 7.5), 20 ul of culture medium diluted in 100 ul of PBST from the wells containing transfectants was added to each microtiter well for 1 hour at 37°C. The wells were then emptied, washed with PBST and either peroxidase-conjugated goat anti human kappa (light chain specific) region antibodies (Sera-Lab) were added and incubated at 37°C for 1 hour, the wells were then emptied, washed with PBST and substrate buffer containing orthophenylenediamine added. Reactions were stopped after a few minutes by the addition of sulphuric acid and absorbance at 492 nm was measured.

EXAMPLE 8: EGF Receptor Radioligand Competition assays

The determination of the affinity constant of the ^{125}I-EGF binding to its receptor by murine R3, chimaeric and mutant by rupture of epitopes T antibodies was performed by a homogeneous Radio Receptor Analysis (RRA) with human placenta microsomal fraction (Macías, A. et al, Interferon y Biotecnología 2: 115-127, 1985).

These chimaeric and mutant by rupture of epitopes T antibodies were assayed using this technique for its ability to bind to EGF-R (figure 5). Both antibodies bound to EGF-R with the same affinity as the original murine antibody (10^{-9}

M), confirming that the correct mouse variable regions had been cloned and the new antibody isotype did not affect binding. Even more, the changes in the mutant antibody did not affect binding to the antigen.

EXAMPLE 9: Immunization of Cercopithecus aethiops monkeys with the murine, chimaeric and VH mutant antibodies.

Three treatment groups with two Cercopithecus aethiops monkeys in each group were immunized with murine R3 mAb, chimaeric R3 antibody and mutant VH R3 antibody, respectively. All the groups were immunized subcutaneously on days 0, 14, 28 and 42, with 2 mg of antibody adsorbed into 5 mg of aluminum hydroxide.

Blood was collected prior to the first immunization and one week later of each immunization, from all the groups, and the serum was obtained from each sample, and kept at -20°C. The titer of antibodies against the murine R3 mAb was determined by an ELISA technique.

Costar plates (Inc, high binding) were coated with murine R3 monoclonal antibody at a concentration of 10 ug/ml in bicarbonate buffer (pH 9.6) and incubated overnight. Thereafter, the plates were washed with PBST, were blocked with the same buffer containing 1% BSA during one hour at room temperature.

The washing step was repeated and 50 ul/well of the different serum dilutions were added. After incubating for 2 hours at 37°C, the plates were washed again and incubated 1 hour at 37°C with alkaline phosphated conjugated goat anti-human total or anti-human IgG Fc region specific antiserum (Sigma, Inc). After washing with PBST the wells were incubated with 50 ul of substrate buffer (1 mg/ml of p-nitrophenylphosphate diluted in diethanolamine buffer (pH 9.8)). Absorbance at 405 nm in an ELISA reader (Organon Teknika, Inc).

A high IgG response to murine R3 antibody was obtained when this antibody was used as immunogen. A lower but still measurable IgG response (1 / 10 000) to the murine R3 antibody was obtained when monkeys were immunized with the chimaeric antibody, contrary to the results obtained with the mutant Vh version (Figure 6). With the mutant VH R3 antibody no response was measurable after two immunizations, and a small response (1 / 10 000) was measured after 4 immunizations.

EXAMPLE 10: Modification of the variable domain sequences of IOR-T1 murine monoclonal antibody to humanize the predicted T-cell antigenic sequences

The variable region sequences of heavy and light chains of IOR-T1 were analyzed for T-cell antigenic sequences. In the variable domain of the heavy chain 3 segments were predicted, they are:

1. FR1 between amino acids 2-21.
2. FR1, CDR1, FR2 between amino acids 29-43.
3. FR4, CDR3 between amino acids 97-111.

FIGURE 7 shows a comparison with the most homologous human sequence and the replacement proposed, which are 5 at the FR1, 2 at the FR2 and 2 at the FR4.

The same procedure with the light chain (Figure 8) rendered the following T-cell antigenic segments:

1. FR3 between amino acids 60-65.
2. FR3, CDR3 between amino acids 79-90.
3. CDR3 between aminoacids 93-95A.

After the analysis we proposed 5 replacement in FR3 at positions: 60, 63, 83, 85 and 87.

EXAMPLE 11: Modification of the variable domain sequences of IOR-CEA1 murine monoclonal antibody to humanize the predicted T-cell antigenic sequences

The variable region sequences of heavy and light chains of IOR-CEA1 were analyzed for T-cell antigenic sequences. In the variable domain of the heavy chain two segments were predicted, they are:

1. FR1 between amino acids 1-16.
2. CDR3 and FR4 between residues 96-110.

FIGURE 9 shows a comparison with the most homologous human sequence and the replacements proposed, which are 7 at the FR1 and 2 at the FR4.

The same analysis with the light chain (Figure 10) rendered the following T-cell antigenic segments:

1. FR1 between amino acids 1-14.

2. CDR2-FR3 between amino acids 55-70.

3. FR3-CDR3-FR4 between residues 74-100.

After the analysis we proposed 4 replacements in FR1 at positions 9, 10, 11 and 13, 11 replacements in FR3 at positions 58, 60, 63, 70, 75, 76, 78, 81, 83, 85 and 87, and 1 replacement in FR4 at position 100.

EXAMPLE 12: Analysis of amphipatic segments in variable regions of immunoglobulin families

The program AMPHI was included as a subroutine in a program written for reading and processing the immunoglobulin sequences from the Kabat Data Base. In processing the sequences the following rearrangements were made:

- Undefined amino acids of type GLX (possible GLN or GLU) were defined as GLN (both GLN and GLU have similar hydrophilicity indexes: -0.22 and -0.64 respectively).
- Undefined amino acids of type ASX (possible ASN or ASP, with hydrophilicity indexes of -0.60 and -0.77) were defined as ASN.
- Other undefined amino acids (empty spaces or "strange" symbols in the sequences were defined as XXX (unknown). The program AMPHI assigns a hydrophilicity value of 0.0 to these amino acids.

Sequences with more than 5 unknown amino acids (XXX) were not included in the analysis.

After this preliminary analysis each sequence was processed by the program AMPHI and the results are presented in the form of tables for each immunoglobulin family.

In tables I to VI the analysis for the six mouse heavy chain families is shown. "Predominant amphipatic regions" (PAR) could be defined at those present in more than 90% of the variable region sequences belonging to each family. For example, comparing the framework one (FR1), a PAR could be defined between the 11 and the 16 amino acid residues for the families I and II, by contrary families III and IV have not amphipatic regions in general from the first amino acid to the 30th. In families V and VI, smaller PARs could be defined from 12-14 and 12-15 residues respectively.

Humanization of the PARs would reduce immunogenicity in patients. The clustering of amphipatic regions in the immunoglobulin variable region frameworks supports the universality of the proposed method, i.e. to humanize these

predicted T-cell epitopes by few point mutations.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Centro de Inmunologia Molecular
(B) STREET: 216 y 15, Atabey Playa, P.O.Box 16040
(C) CITY: Havana
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(F) POSTAL CODE (ZIP): 11600

(ii) TITLE OF INVENTION: Method for obtaining modified immunoglobulins with reduced immunogenicity of murine antibody variable domains, compositions containing them.

(iii) NUMBER OF SEQUENCES: 13

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 95201752.3

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGAAGCTTAG ACCGATGGGG CCTGTTGTTT TG 32

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGAAGCTTGA AGATGGATAC AGTTGGTGCA GC 32

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AGGTSMARCT GCAGSAGTCW GG 22

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TTGAATTCCA GTGATGTTTT GATGACCCA 29

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GACATTCAGC TGACCCA 17

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GTTAGATCTC CAGTTTGGTG CT 22

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GAAGCCCCAG GCTTCTTCAC TTCAGCCCCA GGCTG 35

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GTAAAACGAC GGCCAGT 17

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CAGCCTGGGG CTGAAGTGAA GAAGCCTGGG GCTTCA 36

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ACTGGCCGTC GTTTTAC 17

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 65 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GCAGAGTCCT CAGATCTCAG GCTGCTGAGT TGCATGTAGA CTGTGCTGGT GGATTCGTCT 60

ACCGT 65

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 65 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ACGGTAGACG AATCCACCAG CACAGTCTAC ATGCAACTCA GCAGCCTGAG ATCTGAGGAC 60

TCTGC 65

Claims

1. A method for identifying differences in mammalian species specific amino acid residues within T-cell antigenic sequences in an immunoglobulin, comprising

   a. comparing the framework amino acids of a variable domain of a first mammalian species with the variable domains of a second mammalian species;
   b. determining the subgroups of the second mammalian species to which the first mammalian species most closely corresponds;
   c. determining the second mammalian species sequence which is most similar to the first mammalian species sequence;
   d. identifying amino acid residues of the first mammalian species which differ from the amino acid residues of the second mammalian species, with said amino acids being within T-cell antigenic sequences in the variable region of the immunoglobulins;
   e. identifying only those amino acid residues which are not within a complementarity region or are not directly involved with canonical structures or Vernier zone.

2. The method of claim 1 wherein the first mammalian species is mouse.

3. The method of claim 1 wherein the second mammalian species is human.

4. A method for converting an immunoglobulin having the immunogenicity of a first mammalian species to an antibody having the immunogenicity of a second mammalian species, comprising

replacing the amino acid residues in a first mammalian species framework which differ from the amino acid residues of a second mammalian species with the corresponding amino acid residues from the most similar second mammalian species as identified by the method of claim 1.

5. The method of claim 4 wherein the first mammalian species is mouse.

6. The method of claim 4 wherein the second mammalian species is human.

7. A method comprising

a. preparing a DNA sequence encoding a modified immunoglobulin having specificity for a known antigen wherein some residues within the T-cell antigenic sequences of a first mammalian species which differ from the amino acid residues at the same position of a second mammalian species are replaced with the corresponding amino acid residues from the most similar second mammalian species sequence as identified by the method of claim 1;
b. inserting the sequence into a replicable expression vector operably linked to a suitable promoter compatible with a host cell;
c. transforming the host cell with the vector of b;
d. culturing the host cell;
e. recovering the modified immunoglobulin from the host cell culture.

8. The method of claim 7 wherein the first mammalian species is mouse.

9. The method of claim 7 wherein the second mammalian species is human.

10. A composition comprising a modified immunoglobulin having a specificity for a known antigen.

11. A DNA sequence encoding murine IOR-R3 antibody, which recognizes EGF-R.

12. A DNA sequence encoding modified chimaeric IOR-R3 antibody obtained by methods of claims 1, 4 and 7.

13. A modified chimaeric IOR-R3 antibody exhibiting the antigenicity of human antibody obtained by methods of claims 1, 4 and 7.

14. A modified chimaeric IOR-R3 antibody according to claim 13 with the following point mutations in the framework regions of the heavy chain:
FR1: LEU by VAL at position 11, and VAL by LYS at position 12;
FR3: SER by THR at position 75, THR by SER at position 76, ALA by VAL at position 78 and THR by ARG at position 83.

15. A pharmaceutical composition comprising a modified chimaeric monoclonal antibody according to claim 13 or claim 14.

16. A DNA sequence encoding modified chimaeric IOR-T1 antibody obtained by methods of claims 1, 4 and 7.

17. A modified chimaeric IOR-T1 antibody exhibiting the antigenicity of human antibody obtained by methods of claims 1, 4 and 7.

18. A modified chimaeric IOR-T1 antibody according to claim 17 with the following point mutations in the framework regions of both chains:
Heavy chain:
FR1: LYS by GLN at position 3, VAL by LEU at position 5, GLN by GLU at position 6, LYS by GLN at position 13, LYS by ARG at position 19;
FR2: THR by ALA at position 40, GLU by GLY at position 42;
FR4: THR by LEU at position 108, LEU by VAL at position 109;
Light chain:

FR3: ASP by ALA at position 60, THR by SER at position 63, LEU by PHE at position 83, GLU by VAL at position 85, PHE by TYR at position 87.

19. A pharmaceutical composition comprising a modified chimaeric monoclonal antibody according to claim 17 or claim 18

20. A DNA sequence encoding modified chimaeric IOR-CEA1 antibody obtained by methods of claims 1, 4 and 7.

21. A modified chimaeric IOR-CEA1 antibody exhibiting the antigenicity of human antibody obtained by methods of claims 1, 4 and 7.

22. A modified chimaeric IOR-CEA1 antibody according to claim 21 with the following point mutations in the framework regions of both chains:
Heavy chain:
FR1: PRO by VAL at position 2, LYS by GLN at position 3, LEU by VAL at position 5, GLU by GLN at position 6, GLY by ALA at position 9, ASP by GLU at position 10, GLU by GLY at position 15;
FR4: THR by LEU at position 108, LEU by VAL at position 109; Light chain:
FR1: LYS by SER at position 9, PHE by THR at position 10, SER by LEU at position 11, THR by ALA at position 13;
FR3: VAL by ILE at position 58, ASP by SER at position 60, THR by SER at position 63, ASP by GLU at position 70, ILE by VAL at position 75, SER by ILE at position 76, VAL by LEU at position 78, GLN by ASP at position 81, LEU by PHE at position 83, GLU by THR at position 85, PHE by TYR at position 87;
FR4: ALA by GLN at position 100.

23. A pharmaceutical composition comprising a modified chimaeric monoclonal antibody according to claim 21 or claim 22.

24. The therapeutic use of modified chimaeric monoclonal antibodies according to any one of claims 13, 14, 17, 18, 21 and 22.

25. Use of modified chimaeric antibodies according to any one of claims 13, 14, 17, 18, 21 and 22 for the manufacture of a drug directed to tumors.

26. A modified chimaeric antibody comprising heavy and light chain variable domains derived from a first mammalian species and heavy and light chain constant domains derived from a second mammalian species, wherein the heavy chain variable domain or the light chain variable domain, or both, is modified within T-cell antigenic sequences to adapt the amino acid sequence to the amino acid sequence in the corresponding regions of an antibody derived from said second mammalian species, with the exclusion of modifications in the complementarity determining regions, and modifications in the canonical structures or Vernier zone within the framework regions.

27. A modified chimaeric antibody heavy chain comprising heavy chain variable domains derived from a first mammalian species and heavy chain constant domains derived from a second mammalian species, wherein the heavy chain variable domain is modified within T-cell antigenic sequences to adapt the amino acid sequence to the amino acid sequence in the corresponding heavy chain regions of an antibody derived from said second mammalian species, with the exclusion of modifications in the complementarity determining regions, and modifications in the canonical structures or Vernier zone within the framework regions.

28. A modified chimaeric antibody light chain comprising light chain variable domains derived from a first mammalian species and light chain constant domains derived from a second mammalian species, wherein the light chain variable domain is modified within T-cell antigenic sequences to adapt the amino acid sequence to the amino acid sequence in the corresponding light chain regions of an antibody derived from said second mammalian species, with the exclusion of modifications in the complementarity determining regions, and modifications in the canonical structures or Vernier zone within the framework regions.

FIGURE 1: DEDUCED AMINO ACID SEQUENCES

A VK OF MURINE R3 ANTIBODY

D V L M T Q I P L S L P V S L G D Q A S I S C **R S S Q N I N I V H S N G N T Y L D** W Y L Q K P G Q S P N L L I Y **K V S N R F S** G V P D R F R G S G S G T D F T L K I S R V E A E D L G V Y Y C **F Q Y S H V P W T** F G G G T K L E I K R A

B VH OF MURINE R3 ANTIBODY

Q V Q L Q Q P G A E L V K P G A S V K L S C K A S G Y T F T **N Y Y I Y** W V K Q R P G Q G L E W I G **G I N P T S G G S N F N E K F K T** K A T L T V D E S S T T A Y M Q L S S L T S E D S A V Y Y C T R **Q G L W F D S D G R G F D F** W G Q G T T L T V S S

FIGURE 2: VARIABLE REGION OF THE HEAVY CHAIN OF IOR-R3.

	1	2	3	4	5	6	7	8	9	10	11	12
A	GLN	VAL	GLN	LEU	GLN	GLN	PRO	GLY	ALA	GLU	LEU	VAL
B	GLN	VAL	GLN	LEU	VAL	GLN	SER	GLY	ALA	GLU	VAL	LYS
C	GLN	VAL	GLN	LEU	GLN	GLN	PRO	GLY	ALA	GLU	VAL	LYS

	13	14	15	16	17	18	19	20	21	22	23	24
A	LYS	PRO	GLY	ALA	SER	VAL	LYS	LEU	SER	CYS	LYS	ALA
B	LYS	PRO	GLY	ALA	SER	VAL	LYS	VAL	SER	CYS	LYS	ALA
C	LYS	PRO	GLY	ALA	SER	VAL	LYS	LEU	SER	CYS	LYS	ALA

	25	26	27	28	29	30	31	32	33	34	35	36
A	SER	GLY	TYR	THR	PHE	THR	ASN	TYR	TYR	ILE	TYR	TRP
B	SER	GLY	TYR	THR	PHE	ASN						TRP
C	SER	GLY	TYR	THR	PHE	THR	ASN	TYR	TYR	ILE	TYR	TRP

	37	38	39	40	41	42	43	44	45	46	47	48
A	VAL	LYS	GLN	ARG	PRO	GLY	GLN	GLY	LEU	GLU	TRP	ILE
B	VAL	ARG	GLN	ALA	PRO	GLY	GLN	GLY	LEU	GLU	TRP	MET
C	VAL	LYS	GLN	ARG	PRO	GLY	GLN	GLY	LEU	GLU	TRP	ILE

	49	50	51	52	52A	53	54	55	56	57	58	59
A	GLY	GLY	ILE	ASN	PRO	THR	SER	GLY	GLY	SER	ASN	PHE
B	GLY											
C	GLY	GLY	ILE	ASN	PRO	THR	SER	GLY	GLY	SER	ASN	PHE

	60	61	62	63	64	65	66	67	68	69	70	71
A	ASN	GLU	LYS	PHE	LYS	THR	LYS	ALA	THR	LEU	THR	VAL
B							ARG	VAL	THR	MET	THR	ARG
C	ASN	GLU	LYS	PHE	LYS	THR	LYS	ALA	THR	LEU	THR	VAL

	72	73	74	75	76	77	78	79	80	81	82	82A
A	ASP	GLU	SER	SER	THR	THR	ALA	TYR	MET	GLN	LEU	SER
B	ASP	THR	SER	THR	SER	THR	VAL	TYR	MET	GLU	LEU	SER
C	ASP	GLU	SER	THR	SER	THR	VAL	TYR	MET	GLN	LEU	SER

	82B	82C	83	84	85	86	87	88	89	90	91	92
A	SER	LEU	THR	SER	GLU	ASP	SER	ALA	VAL	TYR	TYR	CYS
B	SER	LEU	ARG	SER	GLU	ASP	THR	ALA	VAL	TYR	TYR	CYS
C	SER	LEU	ARG	SER	GLU	ASP	SER	ALA	VAL	TYR	TYR	CYS

	93	94	95	96	97	98	99	100	100A	100B	100C	100D
A	THR	ARG	GLN	GLY	LEU	TRP	PHE	ASP	SER	ASP	GLY	ARG
B	ALA	ARG										
C	THR	ARG	GLN	GLY	LEU	TRP	PHE	ASP	SER	ASP	GLY	ARG

	100E	100F	101	102	103	104	105	106	107	108	109	110
A	GLY	PHE	ASP	PHE	TRP	GLY	GLN	GLY	THR	THR	LEU	THR
B					TRP	GLY	GLN	GLY	THR	LEU	VAL	THR
C	GLY	PHE	ASP	PHE	TRP	GLY	GLN	GLY	THR	THR	LEU	THR

	111	112	113
A	VAL	SER	SER
B	VAL	SER	SER
C	VAL	SER	SER

FIGURE 3: VARIABLE REGION OF THE LIGHT CHAIN OF IOR-R3.

	1	2	3	4	5	6	7	8	9	10	11	12
A	ASP	VAL	LEU	MET	THR	GLN	ILE	PRO	LEU	SER	LEU	PRO
B	ASP	VAL	VAL	MET	THR	GLN	SER	PRO	LEU	SER	LEU	PRO
C	ASP	VAL	LEU	MET	THR	GLN	ILE	PRO	LEU	SER	LEU	PRO

	13	14	15	16	17	18	19	20	21	22	23	24
A	VAL	SER	LEU	GLY	ASP	GLN	ALA	SER	ILE	SER	CYS	ARG
B	VAL	THR	LEU	GLY	GLN	PRO	ALA	SER	ILE	SER	CYS	
C	VAL	SER	LEU	GLY	ASP	GLN	ALA	SER	ILE	SER	CYS	ARG

	25	26	27	27A	27B	27C	27D	27E	28	29	30	31
A	SER	SER	GLN	ASN	ILE	VAL	HIS	SER	ASN	GLY	ASN	THR
B												
C	SER	SER	GLN	ASN	ILE	VAL	HIS	SER	ASN	GLY	ASN	THR

	32	33	34	35	36	37	38	39	40	41	42	43
A	TYR	LEU	ASP	TRP	TYR	LEU	GLN	LYS	PRO	GLY	GLN	SER
B				TRP	PHE	GLN	GLN	ARG	PRO	GLY	GLN	SER
C	TYR	LEU	ASP	TRP	TYR	LEU	GLN	LYS	PRO	GLY	GLN	SER

	44	45	46	47	48	49	50	51	52	53	54	55
A	PRO	ASN	LEU	LEU	ILE	TYR	LYS	VAL	SER	ASN	ARG	PHE
B	PRO	ARG	ARG	LEU	ILE	TYR						
C	PRO	ASN	LEU	LEU	ILE	TYR	LYS	VAL	SER	ASN	ARG	PHE

	56	57	58	59	60	61	62	63	64	65	66	67
A	SER	GLY	VAL	PRO	ASP	ARG	PHE	ARG	GLY	SER	GLY	SER
B		GLY	VAL	PRO	ASP	ARG	PHE	SER	GLY	SER	GLY	SER
C	SER	GLY	VAL	PRO	ASP	ARG	PHE	ARG	GLY	SER	GLY	SER

	68	69	70	71	72	73	74	75	76	77	78	79
A	GLY	THR	ASP	PHE	THR	LEU	LYS	ILE	SER	ARG	VAL	GLU
B	GLY	THR	ASP	PHE	THR	LEU	LYS	ILE	SER	ARG	VAL	GLU
C	GLY	THR	ASP	PHE	THR	LEU	LYS	ILE	SER	ARG	VAL	GLU

	80	81	82	83	84	85	86	87	88	89	90	91
A	ALA	GLU	ASP	LEU	GLY	VAL	TYR	TYR	CYS	PHE	GLN	TYR
B	ALA	GLU	ASP	VAL	GLY	VAL	TYR	TYR	CYS			
C	ALA	GLU	ASP	LEU	GLY	VAL	TYR	TYR	CYS	PHE	GLN	TYR

	92	93	94	95	96	97	98	99	100	101	102	103
A	SER	HIS	VAL	PRO	TRP	THR	PHE	GLY	GLY	GLY	THR	LYS
B							PHE	GLY	GLN	GLY	THR	LYS
C	SER	HIS	VAL	PRO	TRP	THR	PHE	GLY	GLY	GLY	THR	LYS

	104	105	106	107	108
A	LEU	GLU	ILE	LYS	ARG
B	VAL	GLU	ILE	LYS	ARG
C	LEU	GLU	ILE	LYS	ARG

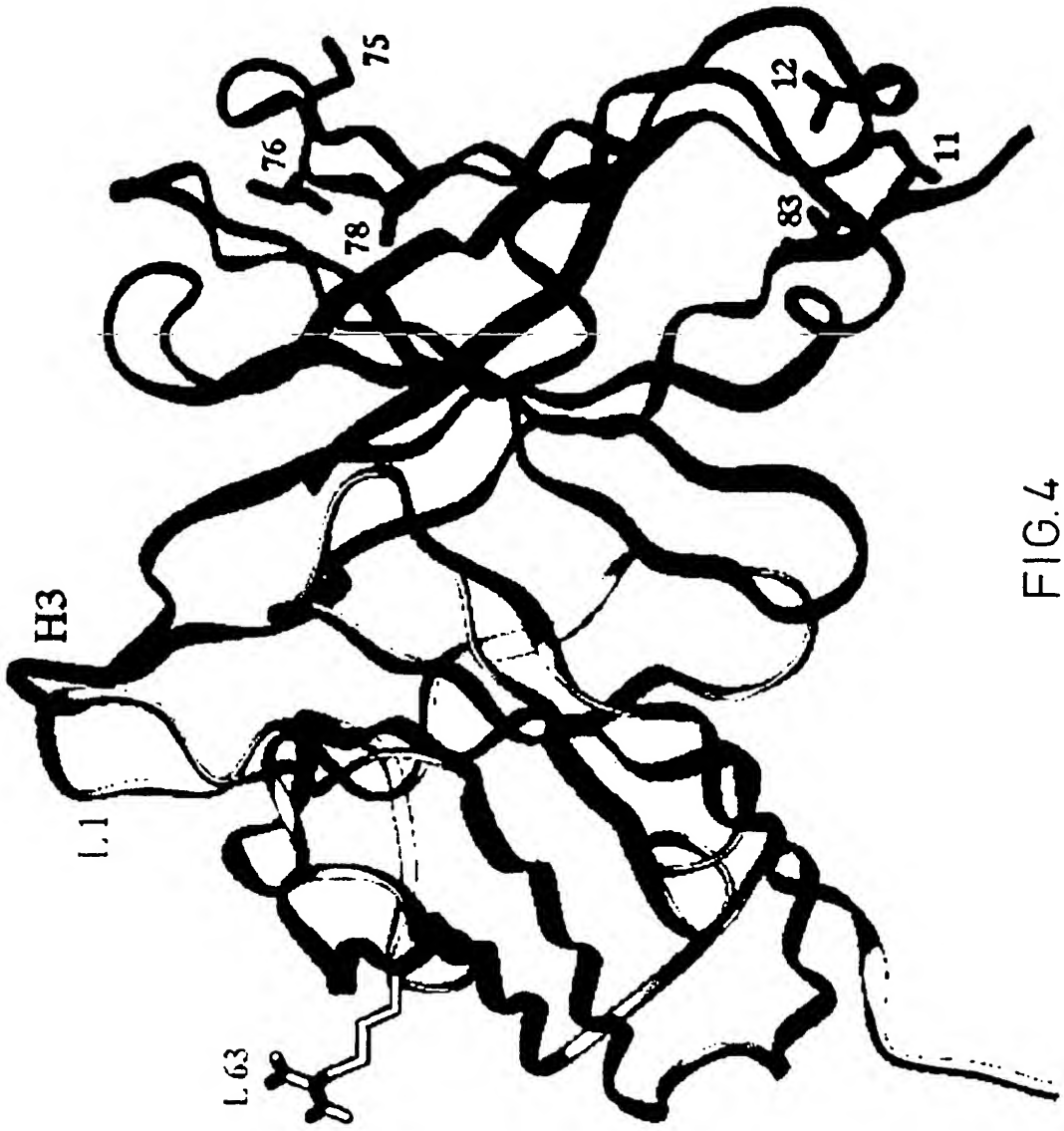

FIG.4

Figure 5

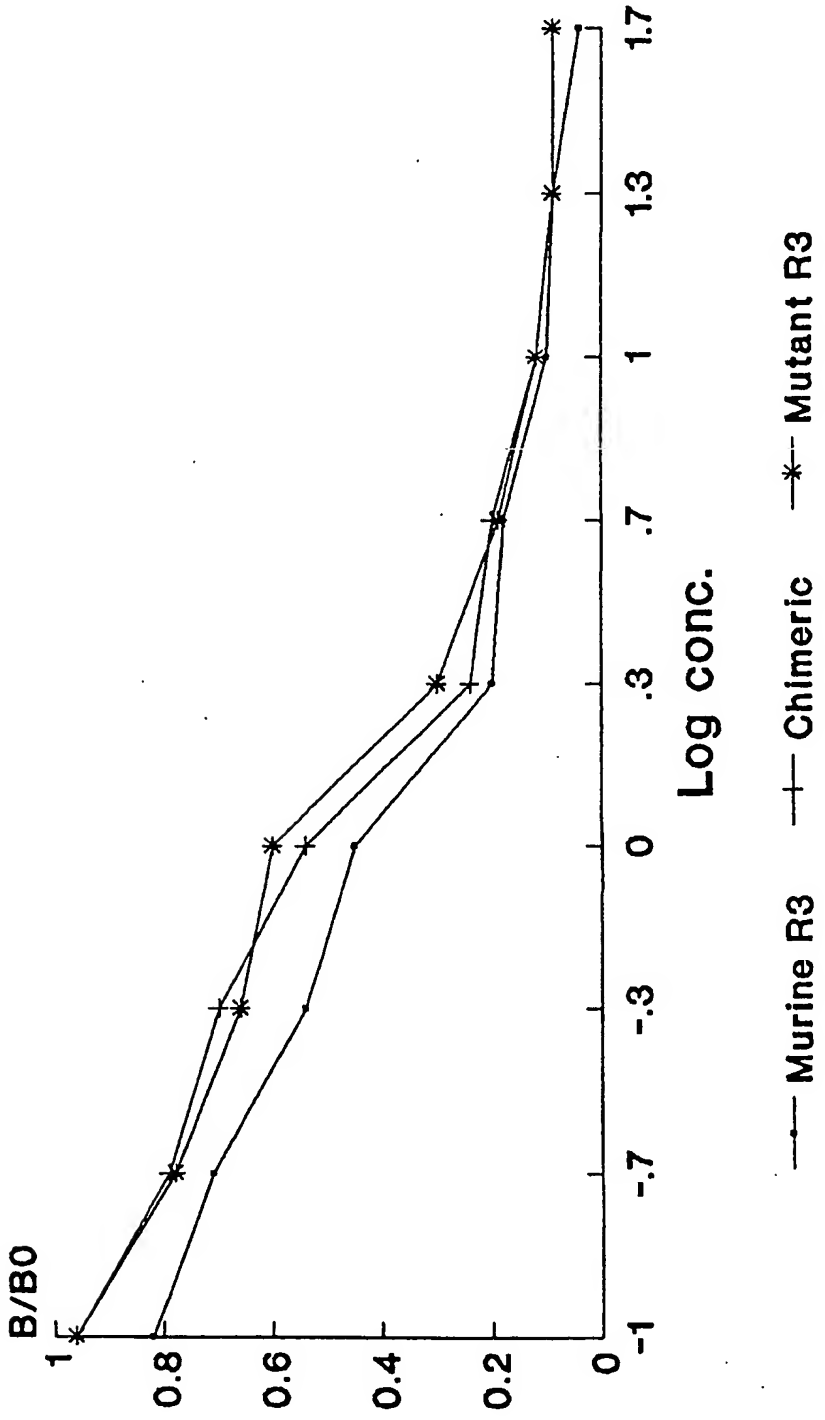
B/B0
1
0.8
0.6
0.4
0.2
0
-1
-.7
-.3
0
.3
.7
1
1.3
1.7
Log conc.
Murine R3
Chimeric
Mutant R3

Figure 6

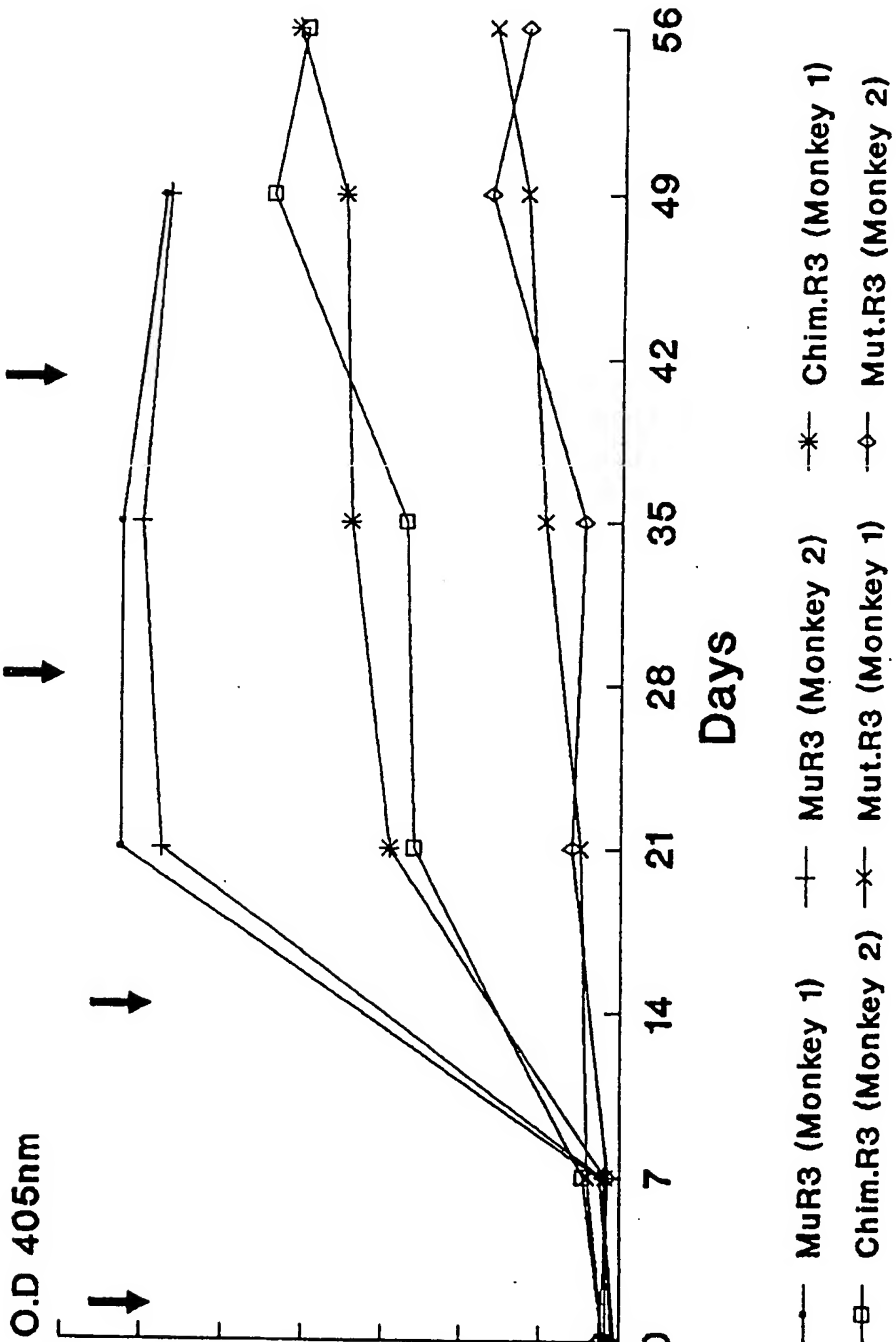
O.D 405nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
7
14
21
28
35
42
49
56
Days
MuR3 (Monkey 1)
MuR3 (Monkey 2)
Chim.R3 (Monkey 1)
Chim.R3 (Monkey 2)
Mut.R3 (Monkey 1)
Mut.R3 (Monkey 2)

FIGURE 7: VARIABLE REGION OF THE HEAVY CHAIN OF IOR-T 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	GLU	VAL	LYS	LEU	VAL	GLN	SER	GLY	GLY	GLY	LEU	VAL
B	GLU	VAL	GLN	LEU	LEU	GLU	SER	GLY	GLY	GLY	LEU	VAL
C	GLU	VAL	GLN	LEU	LEU	GLU	SER	GLY	GLY	GLY	LEU	VAL

	13	14	15	16	17	18	19	20	21	22	23	24
A	LYS	PRO	GLY	GLY	SER	LEU	LYS	LEU	SER	CYS	ALA	ALA
B	GLN	PRO	GLY	GLY	SER	LEU	ARG	LEU	SER	CYS	ALA	ALA
C	GLN	PRO	GLY	GLY	SER	LEU	ARG	LEU	SER	CYS	ALA	ALA

	25	26	27	28	29	30	31	32	33	34	35	36
A	SER	GLY	PHE	LYS	PHE	SER	ARG	TYR	ALA	MET	SER	TRP
B	SER	GLY	PHE	THR	PHE	SER						TRP
C	SER	GLY	PHE	LYS	PHE	SER	ARG	TYR	ALA	MET	SER	TRP

	37	38	39	40	41	42	43	44	45	46	47	48
A	VAL	ARG	GLN	THR	PRO	GLU	LYS	ARG	LEU	GLU	TRP	VAL
B	VAL	ARG	GLN	ALA	PRO	GLY	LYS	GLY	LEU	GLU	TRP	VAL
C	VAL	ARG	GLN	ALA	PRO	GLY	LYS	ARG	LEU	GLU	TRP	VAL

	49	50	51	52	52A	53	54	55	56	57	58	59
A	ALA	THR	ILE	SER	SER	GLY	GLY	SER	SER	HIS	LEU	LEU
B	SER											
C	SER	THR	ILE	SER	SER	GLY	GLY	SER	SER	HIS	LEU	LEU

	60	61	62	63	64	65	66	67	68	69	70	71
A	SER	ARG	GLN	CYS	GLU	GLY	ARG	PHE	THR	ILE	SER	ARG
B							ARG	PHE	THR	ILE	SER	ARG
C	SER	ARG	GLN	CYS	GLU	GLY	ARG	PHE	THR	ILE	SER	ARG

	72	73	74	75	76	77	78	79	80	81	82	82A
A	ASP	ASN	VAL	LYS	ASN	THR	LEU	TYR	LEU	GLN	MET	SER
B	ASP	ASN	SER	LYS	ASN	THR	LEU	TYR	LEU	GLN	MET	ASN
C	ASP	ASN	VAL	LYS	ASN	THR	LEU	TYR	LEU	GLN	MET	SER

	82B	82C	83	84	85	86	87	88	89	90	91	92
A	SER	LEU	ARG	SER	GLU	ASP	THR	ALA	MET	TYR	TYR	CYS
B	SER	LEU	ARG	ALA	GLU	ASP	THR	ALA	VAL	TYR	TYR	CYS
C	SER	LEU	ARG	SER	GLU	ASP	THR	ALA	MET	TYR	TYR	CYS

	93	94	95	96	97	98	99	100	100A	100B	101	102
A	ALA	ARG	ARG	ASP	TYR	ASP	LEU	ASP	TYR	PHE	ALA	SER
B	ALA	LYS										
C	ALA	ARG	ARG	ASP	TYR	ASP	LEU	ASP	TYR	PHE	ALA	SER

	103	104	105	106	107	108	109	110	111	112	113
A	TRP	GLY	GLN	GLY	THR	THR	LEU	THR	VAL	SER	SER
B	TRP	GLY	GLN	GLY	THR	LEU	VAL	THR	VAL	SER	SER
C	TRP	GLY	GLN	GLY	THR	LEU	VAL	THR	VAL	SER	SER

FIGURE 8: VARIABLE REGION OF THE LIGHT CHAIN OF IOR-T 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	ASP	ILE	VAL	MET	THR	GLN	ASP	GLN	LYS	PHE	MET	SER
B	GLU	ILE	VAL	MET	THR	GLN	SER	PRO	ALA	THR	LEU	SER
C	ASP	ILE	VAL	MET	THR	GLN	ASP	GLN	LYS	PHE	MET	SER

	13	14	15	16	17	18	19	20	21	22	23	24
A	THR	SER	VAL	GLY	ASP	ARG	VAL	SER	VAL	THR	CYS	LYS
B	VAL	SER	PRO	GLY	GLU	ARG	ALA	THR	LEU	SER	CYS	
C	THR	SER	VAL	GLY	ASP	ARG	VAL	SER	VAL	THR	CYS	LYS

	25	26	27	28	29	30	31	32	33	34	35	36
A	ALA	SER	GLN	ASN	ALA	GLY	THR	ASN	VAL	ALA	TRP	TYR
B											TRP	TYR
C	ALA	SER	GLN	ASN	ALA	GLY	THR	ASN	VAL	ALA	TRP	TYR

	37	38	39	40	41	42	43	44	45	46	47	48
A	GLN	GLN	LYS	PRO	GLY	GLN	SER	PRO	LYS	ALA	LEU	ILE
B	GLN	GLN	LYS	PRO	GLY	GLN	PRO	PRO	ARG	LEU	LEU	ILE
C	GLN	GLN	LYS	PRO	GLY	GLN	SER	PRO	LYS	ALA	LEU	ILE

	49	50	51	52	53	54	55	56	57	58	59	60
A	TYR	SER	ALA	SER	SER	ARG	ASN	SER	GLY	VAL	PRO	ASP
B	TYR								GLY	ILE	PRO	ALA
C	TYR	SER	ALA	SER	SER	ARG	ASN	SER	GLY	VAL	PRO	ALA

	61	62	63	64	65	66	67	68	69	70	71	72
A	ARG	PHE	THR	GLY	SER	GLY	SER	GLY	THR	ASP	PHE	THR
B	ARG	PHE	SER	GLY	SER	GLY	SER	GLY	THR	GLU	PHE	THR
C	ARG	PHE	SER	GLY	SER	GLY	SER	GLY	THR	ASP	PHE	THR

	73	74	75	76	77	78	79	80	81	82	83	84
A	LEU	THR	ILE	SER	ASN	VAL	GLN	SER	GLU	ASP	LEU	ALA
B	LEU	THR	ILE	SER	ARG	LEU	GLN	SER	GLU	ASP	PHE	ALA
C	LEU	THR	ILE	SER	ASN	VAL	GLN	SER	GLU	ASP	PHE	ALA

	85	86	87	88	89	90	91	92	93	94	95	95A
A	GLU	TYR	PHE	CYS	GLN	GLN	TYR	ASN	SER	TYR	PRO	LEU
B	VAL	TYR	TYR	CYS								
C	VAL	TYR	TYR	CYS	GLN	GLN	TYR	ASN	SER	TYR	PRO	LEU

	96	97	98	99	100	101	102	103	104	105	106	107
A	VAL	THR	PHE	GLY	ALA	GLY	THR	LYS	LEU	GLU	LEU	LYS
B			PHE	GLY	GLN	GLY	THR	ARG	VAL	GLU	ILE	LYS
C	VAL	THR	PHE	GLY	ALA	GLY	THR	LYS	LEU	GLU	LEU	LYS

	108	109
A	ARG	ALA
B	ARG	GLU
C	ARG	ALA

FIGURE 9: VARIABLE REGION OF THE HEAVY CHAIN OF IOR-CEA-1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	GLN	PRO	LYS	LEU	LEU	GLU	SER	GLY	GLY	ASP	LEU	VAL
B	GLN	VAL	GLN	LEU	VAL	GLN	SER	GLY	ALA	GLU	VAL	LYS
C	GLN	VAL	GLN	LEU	VAL	GLN	SER	GLY	ALA	GLU	LEU	VAL

	13	14	15	16	17	18	19	20	21	22	23	24
A	LYS	PRO	GLU	ALA	SER	LEU	ASN	CYS	SER	CYS	ALA	VAL
B	LYS	PRO	GLY	ALA	SER	LEU	LYS	VAL	SER	CYS	LYS	ALA
C	LYS	PRO	GLY	ALA	SER	LEU	ASN	CYS	SER	CYS	ALA	VAL

	25	26	27	28	29	30	31	32	33	34	35	36
A	SER	GLY	PHE	PRO	PHE	ASN	ARG	TYR	ALA	MET	SER	TRP
B	SER	GLY	TYR	THR	PHE	THR						TRP
C	SER	GLY	PHE	PRO	PHE	ASN	ARG	TYR	ALA	MET	SER	TRP

	37	38	39	40	41	42	43	44	45	46	47	48
A	VAL	LEU	GLN	THR	PRO	GLU	LYS	ARG	LEU	GLU	TRP	VAL
B	VAL	ARG	GLN	ALA	PRO	GLY	GLN	ARG	LEU	GLU	TRP	MET
C	VAL	LEU	GLN	THR	PRO	GLU	LYS	ARG	LEU	GLU	TRP	VAL

	49	50	51	52	52A	53	54	55	56	57	58	59
A	ALA	PHE	ILE	SER	SER	ASP	ASP	GLY	ILE	ALA	TYR	TYR
B	GLY											
C	ALA	PHE	ILE	SER	SER	ASP	ASP	GLY	ILE	ALA	TYR	TYR

	60	61	62	63	64	65	66	67	68	69	70	71
A	ALA	GLU	SER	LYS	GLY	TYR	ARG	PHE	THR	ILE	SER	ARG
B							ARG	VAL	THR	ILE	THR	ARG
C	ALA	GLU	SER	LYS	GLY	TYR	ARG	PHE	THR	ILE	SER	ARG

	72	73	74	75	76	77	78	79	80	81	82	82A
A	ASP	ASN	ALA	LYS	ASN	ILE	LEU	TYR	LEU	GLN	MET	SER
B	ASP	THR	SER	ALA	SER	THR	ALA	TYR	MET	GLU	LEU	SER
C	ASP	ASN	ALA	LYS	ASN	THR	LEU	TYR	LEU	GLN	MET	SER

	82B	82C	83	84	85	86	87	88	89	90	91	92
A	SER	LEU	ARG	SER	GLN	ASP	THR	ALA	MET	TYR	TYR	CYS
B	SER	LEU	ARG	SER	GLU	ASP	THR	ALA	VAL	TYR	TYR	CYS
C	SER	LEU	ARG	SER	GLN	ASP	THR	ALA	VAL	TYR	TYR	CYS

	93	94	95	96	97	98	99	100	100A	100B	100C	101
A	ALA	ARG	VAL	TYR	TYR	TYR	GLY	SER	SER	TYR	PHE	ASP
B	ALA	ARG										
C	ALA	ARG	VAL	TYR	TYR	TYR	GLY	SER	SER	TYR	PHE	ASP

	102	103	104	105	106	107	108	109	110	111	112	113
A												
B	TYR	TRP	GLY	GLN	GLY	THR	THR	LEU	THR	VAL	SER	SER
C		TRP	GLY	GLU	GLY	THR	LEU	VAL	THR	VAL	SER	SER
	TYR	TRP	GLY	GLN	GLY	THR	LEU	VAL	THR	VAL	SER	SER

FIGURE 10:VARIABLE REGION OF THE LIGHT OF IOR-CEA 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	ASP	ILE	GLN	MET	THR	GLN	SER	PRO	LYS	PHE	SER	SER
B	ASP	ILE	GLN	MET	THR	GLN	SER	PRO	SER	THR	LEU	SER
C	ASP	ILE	GLN	MET	THR	GLN	SER	PRO	SER	THR	LEU	SER

	13	14	15	16	17	18	19	20	21	22	23	24
A	THR	SER	VAL	GLY	ASP	ARG	VAL	SER	VAL	THR	CYS	LYS
B	ALA	SER	VAL	GLY	ASP	SER	ILE	THE	ILE	THR	CYS	
C	ALA	SER	VAL	GLY	ASP	ARG	VAL	SER	VAL	THR	CYS	LYS

	25	26	27	28	29	30	31	32	33	34	35	36
A	ALA	SER	GLN	ASN	ALA	GLY	ILE	ASN	VAL	ALA	TRP	TYR
B											TRP	PHE
C	ALA	SER	GLN	ASN	ALA	GLY	ILE	ASN	VAL	ALA	TRP	TYR

	37	38	39	40	41	42	43	44	45	46	47	48
A	GLN	GLN	LYS	PRO	GLY	GLN	SER	PRO	LYS	ALA	LEU	ILE
B	GLN	GLN	LYS	PRO	GLY	LYS	ALA	PRO	ASN	VAL	LEU	ILE
C	GLN	GLN	LYS	PRO	GLY	GLN	SER	PRO	LYS	ALA	LEU	ILE

	49	50	51	52	53	54	55	56	57	58	59	60
A	TYR	SER	ALA	SER	SER	ARG	ASN	SER	GLY	VAL	PRO	ASP
B	TYR								GLY	ILE	PRO	SER
C	TYR	SER	ALA	SER	SER	ARG	ASN	SER	GLY	ILE	PRO	SER

	61	62	63	64	65	66	67	68	69	70	71	72
A	ARG	PHE	THR	GLY	SER	GLY	SER	GLY	THR	ASP	PHE	THR
B	ARG	PHE	SER	GLY	SER	GLY	SER	GLY	THR	GLU	PHE	THR
C	ARG	PHE	SER	GLY	SER	GLY	SER	GLY	THR	GLU	PHE	THR

	73	74	75	76	77	78	79	80	81	82	83	84
A	LEU	THR	ILE	SER	ASN	VAL	GLN	SER	GLN	ASP	LEU	ALA
B	LEU	THR	VAL	ILE	ASN	LEU	GLN	SER	ASP	ASP	PHE	ALA
C	LEU	THR	VAL	ILE	ASN	LEU	GLN	SER	ASP	ASP	PHE	ALA

	85	86	87	88	89	90	91	92	93	94	95	95A
A	GLU	TYR	PHE	CYS	GLN	GLN	TYR	ASN	SER	TYR	PRO	LEU
B	THR	TYR	TYR	CYS								
C	THR	TYR	TYR	CYS	GLN	GLN	TYR	ASN	SER	TYR	PRO	LEU

	96	97	98	99	100	101	102	103	104	105	106	107
A	VAL	THR	PHE	GLY	ALA	GLY	THR	LYS	LEU	GLN	LEU	LYS
B			PHE	GLY	GLN	GLY	THR	LYS	VAL	LEU	ILE	LYS
C	VAL	THR	PHE	GLY	GLN	GLY	THR	LYS	LEU	GLN	LEU	LYS

	108	109
A	ARG	THR
B	ARG	THR
C	ARG	THR

Table 1

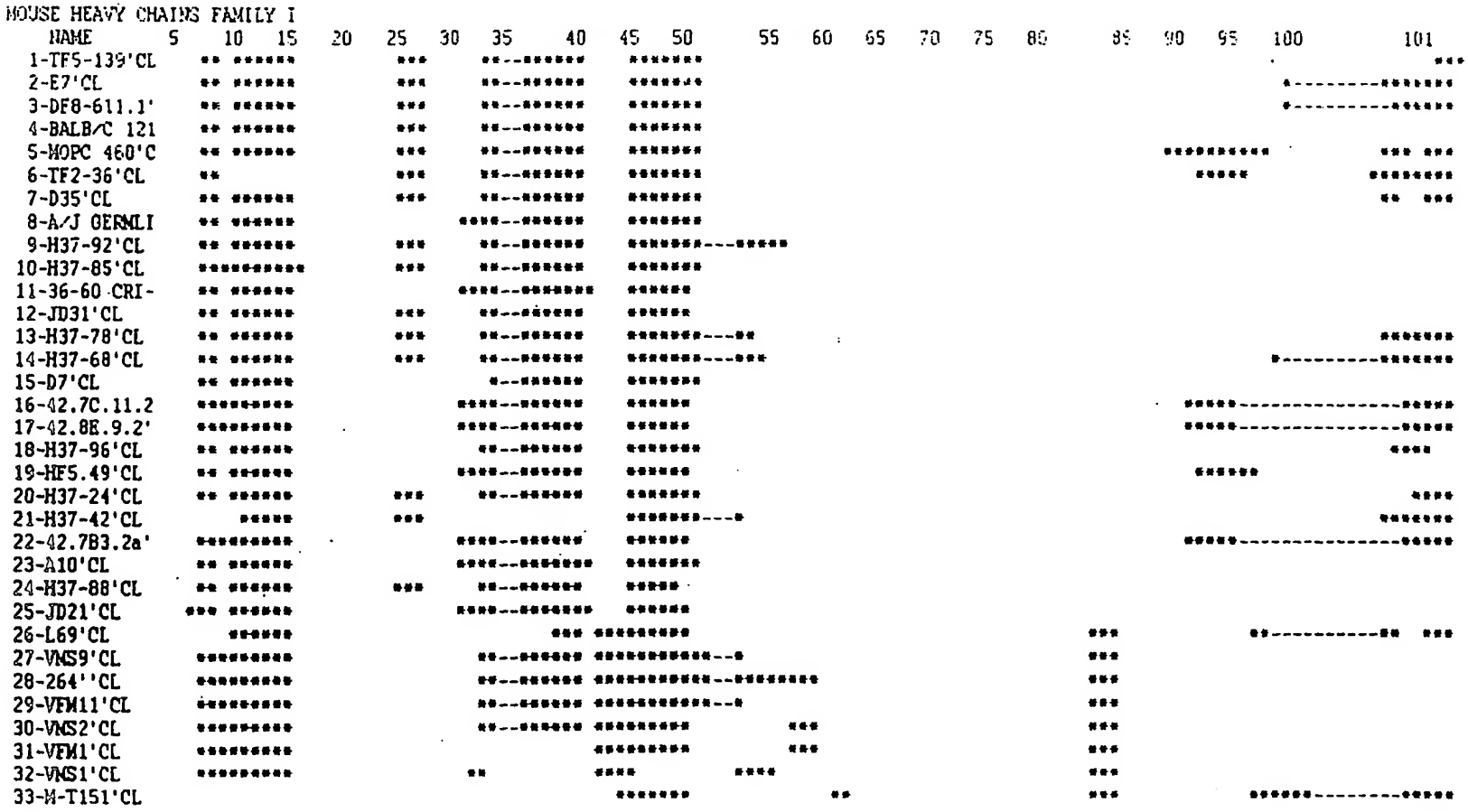

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Table 2

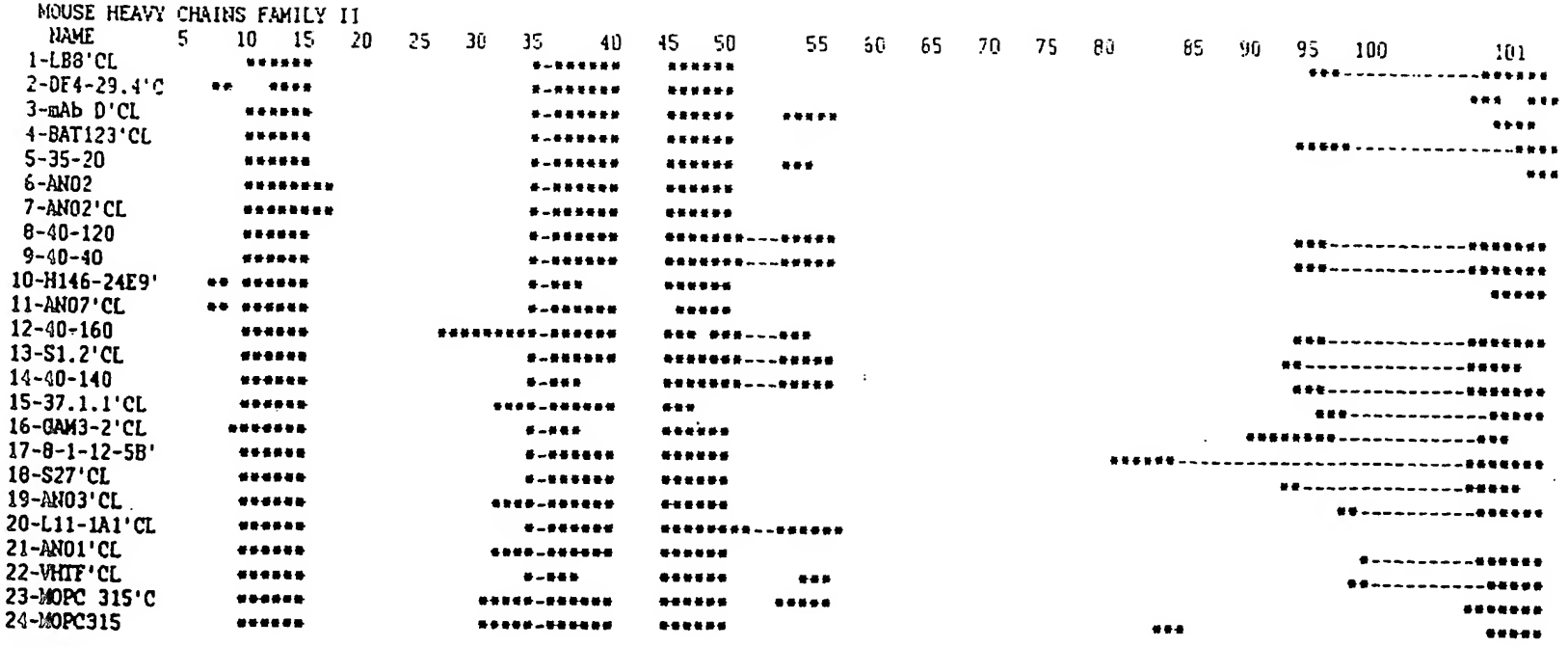

MOUSE HEAVY CHAINS FAMILY II
NAME
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
101
1-LB8'CL
2-DF4-29.4'C
3-mAb D'CL
4-BAT123'CL
5-35-20
6-AN02
7-AN02'CL
8-40-120
9-40-40
10-H146-24E9'
11-AN07'CL
12-40-160
13-S1.2'CL
14-40-140
15-37.1.1'CL
16-GAM3-2'CL
17-8-1-12-5B'
18-S27'CL
19-AN03'CL
20-L11-1A1'CL
21-AN01'CL
22-VHTF'CL
23-MOPC 315'C
24-MOPC315

Table 3

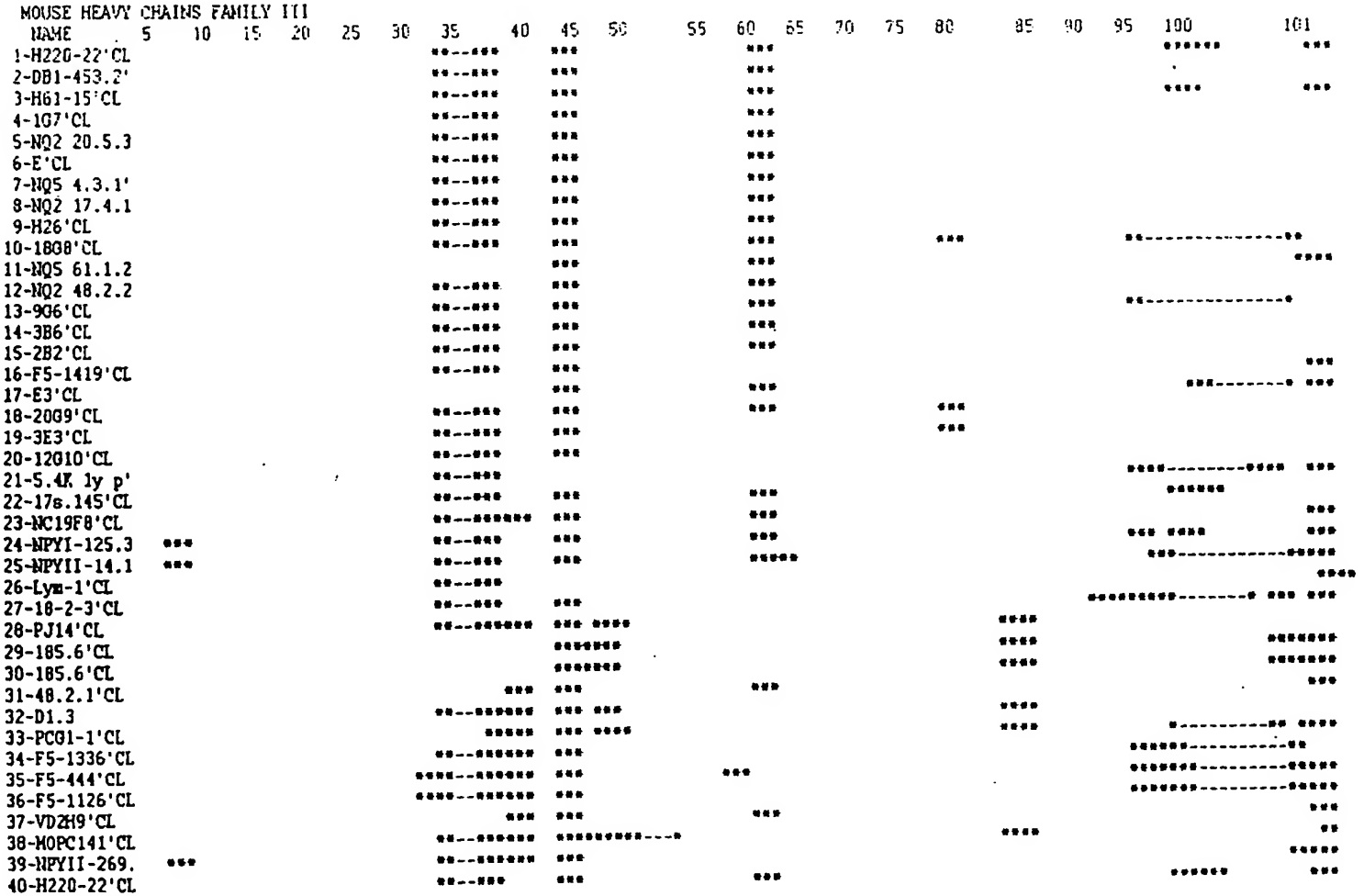
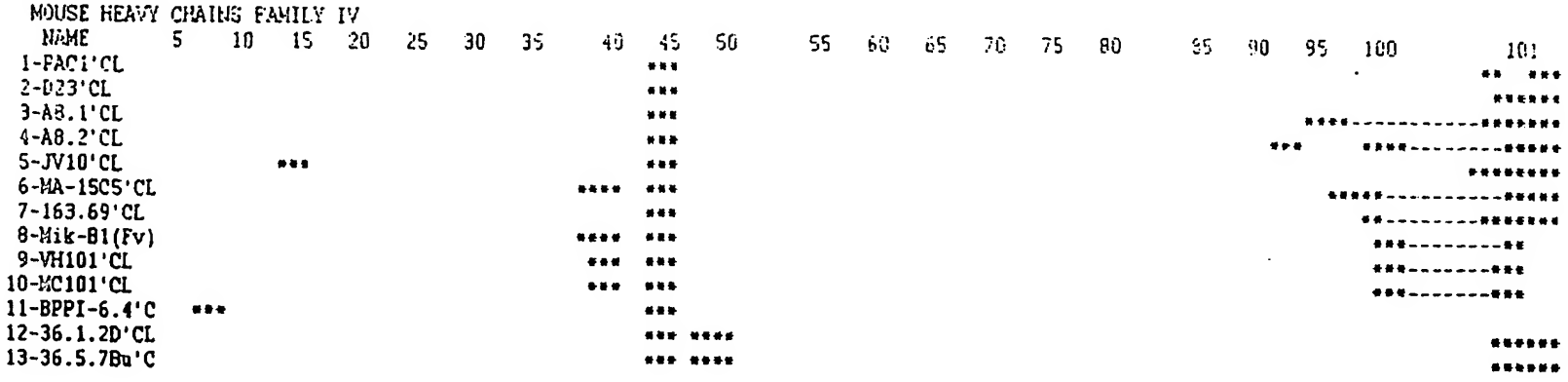

MOUSE HEAVY CHAINS FAMILY III
NAME
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 101
1-H220-22'CL
2-DB1-453.2'
3-H61-15'CL
4-1G7'CL
5-NQ2 20.5.3
6-E'CL
7-NQ5 4.3.1'
8-NQ2 17.4.1
9-H26'CL
10-1BG8'CL
11-NQ5 61.1.2
12-NQ2 48.2.2
13-9G6'CL
14-3B6'CL
15-2B2'CL
16-F5-1419'CL
17-E3'CL
18-2G9'CL
19-3E3'CL
20-12G10'CL
21-5.4K 1y p'
22-17a.145'CL
23-NC19F8'CL
24-NPYI-125.3
25-NPYII-14.1
26-Lym-1'CL
27-18-2-3'CL
28-PJ14'CL
29-18S.6'CL
30-18S.6'CL
31-48.2.1'CL
32-D1.3
33-PCG1-1'CL
34-F5-1336'CL
35-F5-444'CL
36-F5-1126'CL
37-VDZH9'CL
38-HOPC141'CL
39-NPYII-269.
40-H220-22'CL

Table 4



EP 0 699 755 A2

Table 5

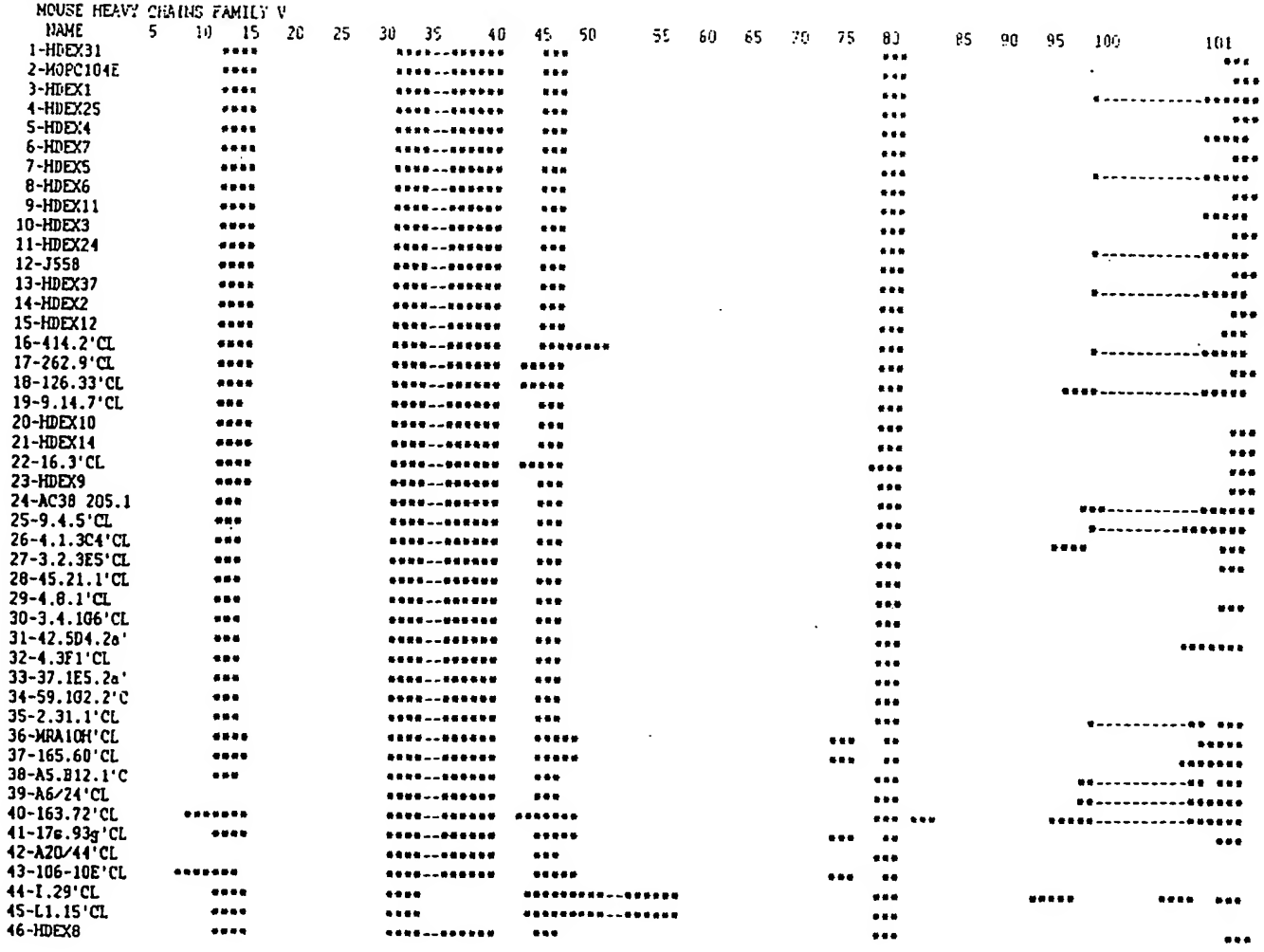
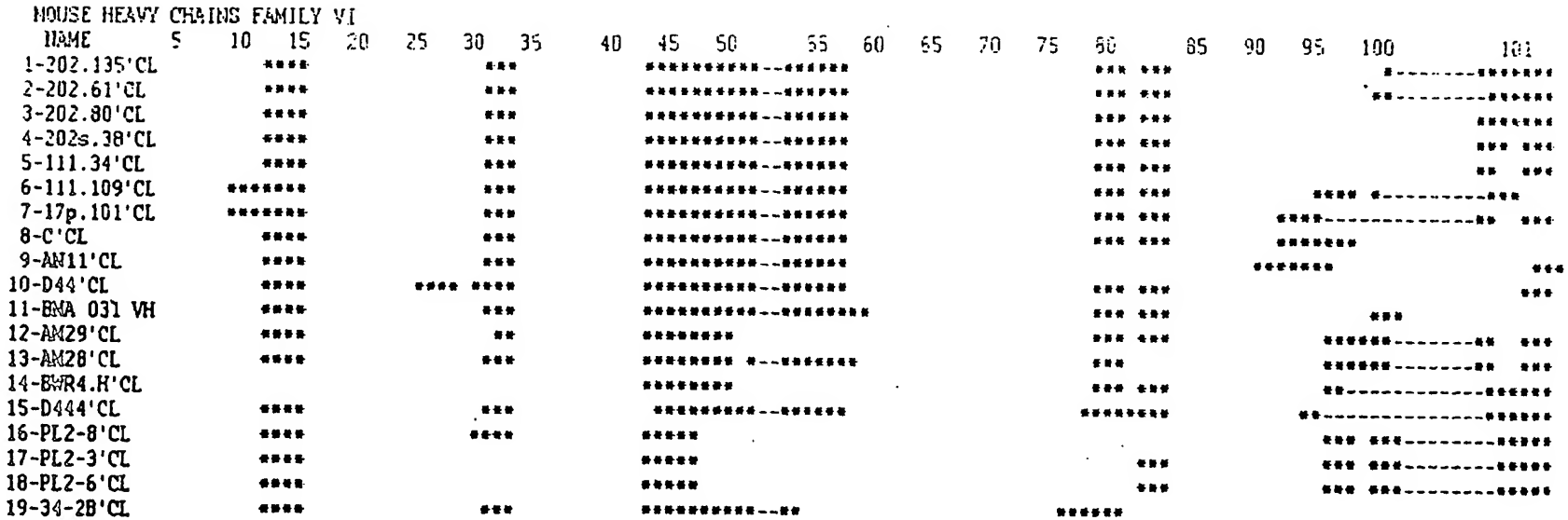

MOUSE HEAVY CHAINS FAMILY V
NAME
1-HDEX31
2-MOPC104E
3-HDEX1
4-HDEX25
5-HDEX4
6-HDEX7
7-HDEX5
8-HDEX6
9-HDEX11
10-HDEX3
11-HDEX24
12-J558
13-HDEX37
14-HDEX2
15-HDEX12
16-414.2'CL
17-262.9'CL
18-126.33'CL
19-9.14.7'CL
20-HDEX10
21-HDEX14
22-16.3'CL
23-HDEX9
24-AC38 205.1
25-9.4.5'CL
26-4.1.3C4'CL
27-3.2.3E5'CL
28-45.21.1'CL
29-4.8.1'CL
30-3.4.1G6'CL
31-42.5D4.2a'
32-4.3F1'CL
33-37.1E5.2a'
34-59.1G2.2'C
35-2.31.1'CL
36-MRA10H'CL
37-165.60'CL
38-A5.B12.1'C
39-A6/24'CL
40-163.72'CL
41-17a.93g'CL
42-A20/44'CL
43-106-10E'CL
44-I.29'CL
45-L1.15'CL
46-HDEX8

Table 6



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(54) Method for obtaining modified immunoglobulins with reduced immunogenicity of murine antibody variable domains, compositions containing them

(57) Modified chimaeric antibodies, and antibody heavy and light chains, which comprise variable domains derived from a first mammalian species, usually mouse, and constant domains from a second mammalian species, usually human. Modification concerns the variable domains, in particular the framework regions of the variable domains. The modifications are made only in T-cell antigenic structures present in framework regions, and do not cover canonical structures or Vernier zone. The modifications adapt the amino acid sequences concerned to those occurring in corresponding antibodies derived from said second mammalian species. Thus, the modified chimaeric antibodies retain the original antigen recognition and binding properties but become less immunogenic to said second mammalian species, which improves their therapeutical utility with said second mammalian species. Recombinant DNA technology may be used to construct and produce the modified chimaeric antibodies.

FIGURE 1: DEDUCED AMINO ACID SEQUENCES

A VK OF MURINE R3 ANTIBODY

D V L M T Q I P L S L P V S L G D Q A S I S C R S S Q
N I V H S N G N T Y L E W Y L Q K P G Q S P N L L
I Y K V S N R F S G V P D R F S G S G S G T D F T L K
I S R V E A E D L G V Y Y C F Q Y S H V P W T F G G G
T K L E I K R A

B VH OF MURINE R3 ANTIBODY

Q V Q L Q Q P G A E L V K P G A S V K L S C K A S G Y
T F T N Y W I H W V K Q R P G Q G L E W I G E I N P S
S G G S N F N E K F K S K A T L T V D K S S T T A Y M
Q L S S L T S E D S A V Y Y C T R S G L W F D S P G R
G T D Y W G Q G T T L T V S S

EP 0 699 755 A3

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 95 20 1752

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,X	MOLECULAR IMMUNOLOGY, vol.28, no.4/5, 1, OXFORD, GB pages 489 - 498 E. PADLAN 'A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties.' * the whole document * ---	1-10, 26-28	C12N15/13 C07K16/46 G01N33/68 A61K39/395
D,X	EP-A-0 519 596 (MERCK & CO., INC. & NIH) 23 December 1992 * example * * claims * ---	1-10, 26-28	
X	WO-A-93 11794 (XOMA CORP.) 24 June 1993 * examples 1-4 * * figure 2 * * claims * --- -/--	1-10, 26-28	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C12N
C07K
G01N
A61K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	3 November 1995	NOOIJ, F

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number EP 95 20 1752

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	EP-A-0 592 106 (IMMUNOGEN INC.) 13 April 1994 * page 7, line 10 - page 9, line 27 * * example 1 * * figure 2 * * claims *	1-10, 26-28	
X	PROTEIN ENGINEERING, vol.7, no.6, 4, OXFORD, GB pages 805 - 814 G. STUDNICKA ET AL. 'Human-engineered monoclonal antibodies retain full specific binding activity by preserving non-CDR complementarity-modulating residues.' * the whole document *	1-10, 26-28	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
A	PROTEIN ENGINEERING, vol.4, no.7, 1, OXFORD, GB pages 773 - 783 C. KETTLEBOROUGH ET AL. 'Humanization of a mouse monoclonal antibody by CDR-grafting: the importance of framework residues on loop conformation.' * abstract *	1-15, 24-28	
A	JOURNAL OF MOLECULAR BIOLOGY, vol.235, no.1, 7 January 1994, LONDON, GB pages 53 - 60 A. CORTI ET AL. 'Idiotope determining regions of a mouse monoclonal antibody and its humanized versions.' * the whole document *	1-10, 26-28	
A	EP-A-0 586 002 (CENTRO DE IMMUNOLOGIA MOLECULAR) 9 March 1994 * claims *	12-15	

EPO FORM 1503 03.82 (P04C10)

European Patent Office

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,
namely:

see sheet -B-

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respects of which search fees have been paid,

namely claims:

☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims,

namely claims: 1-9,12-28

European Patent Office

EP 95 20 1752 -B-

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims 1-9 and 12-28:

   Method for obtaining modified immunoglobulins with reduced immunogenicity of murine antibody variable domains, compositions containing them

2. Claim 10:

   Composition comprising a modified immunoglobulin having a specificity for a known antigen

3. Claim 11:

   DNA sequence encoding murine IOR-R3 antibody which recognizes EGF-R

EP 95 20 1752

-C-

Remark: Although claim 24 is directed to a method of treatment of the human/animal body (Art. 52(4) EPC) the search has been carried out and based on the alleged effects of the compound/composition